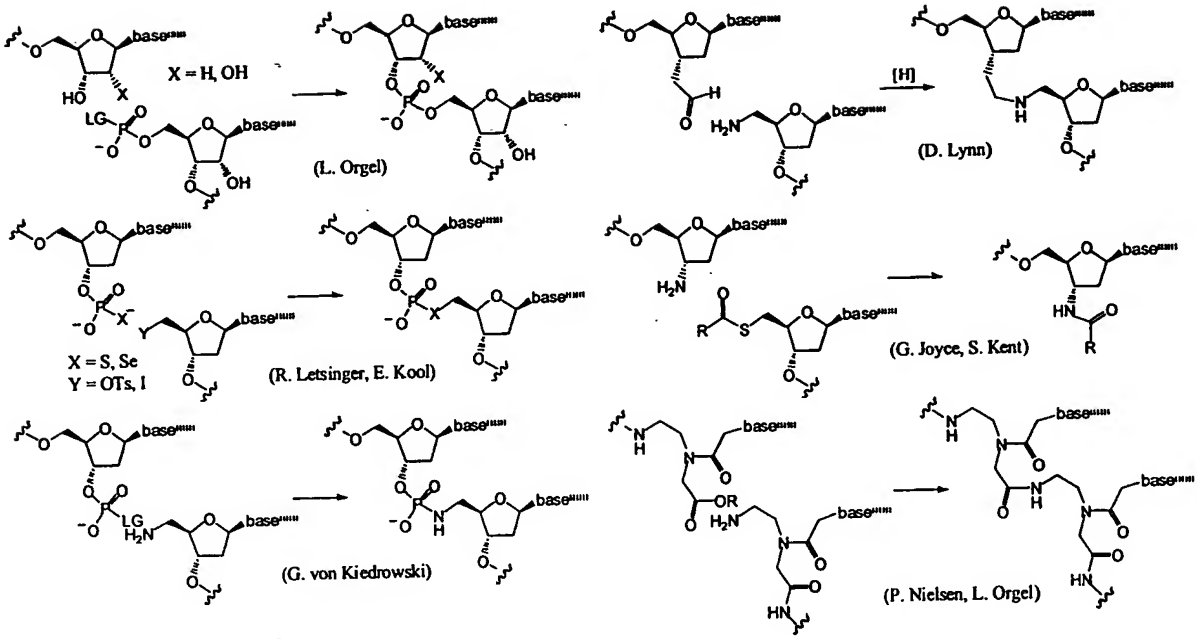
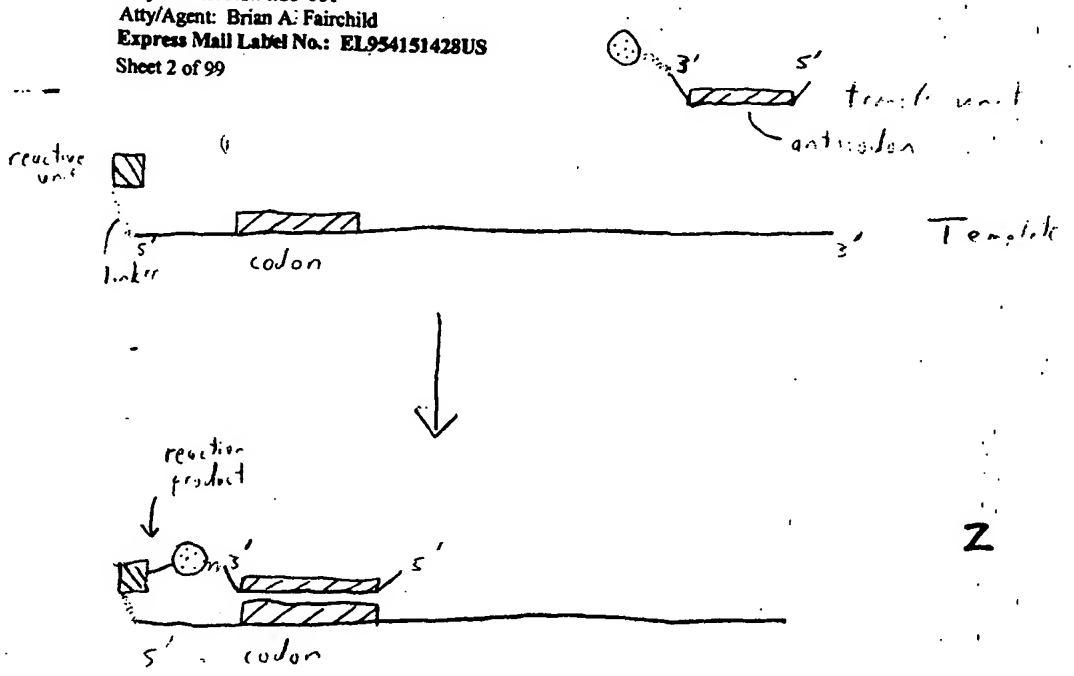
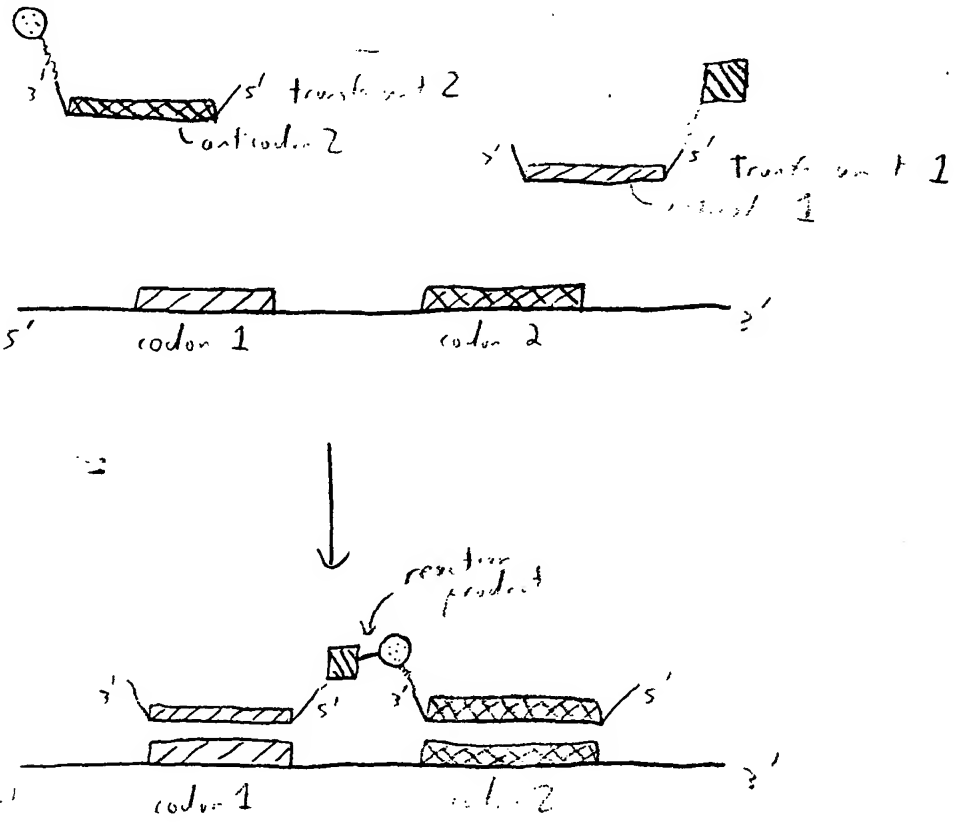
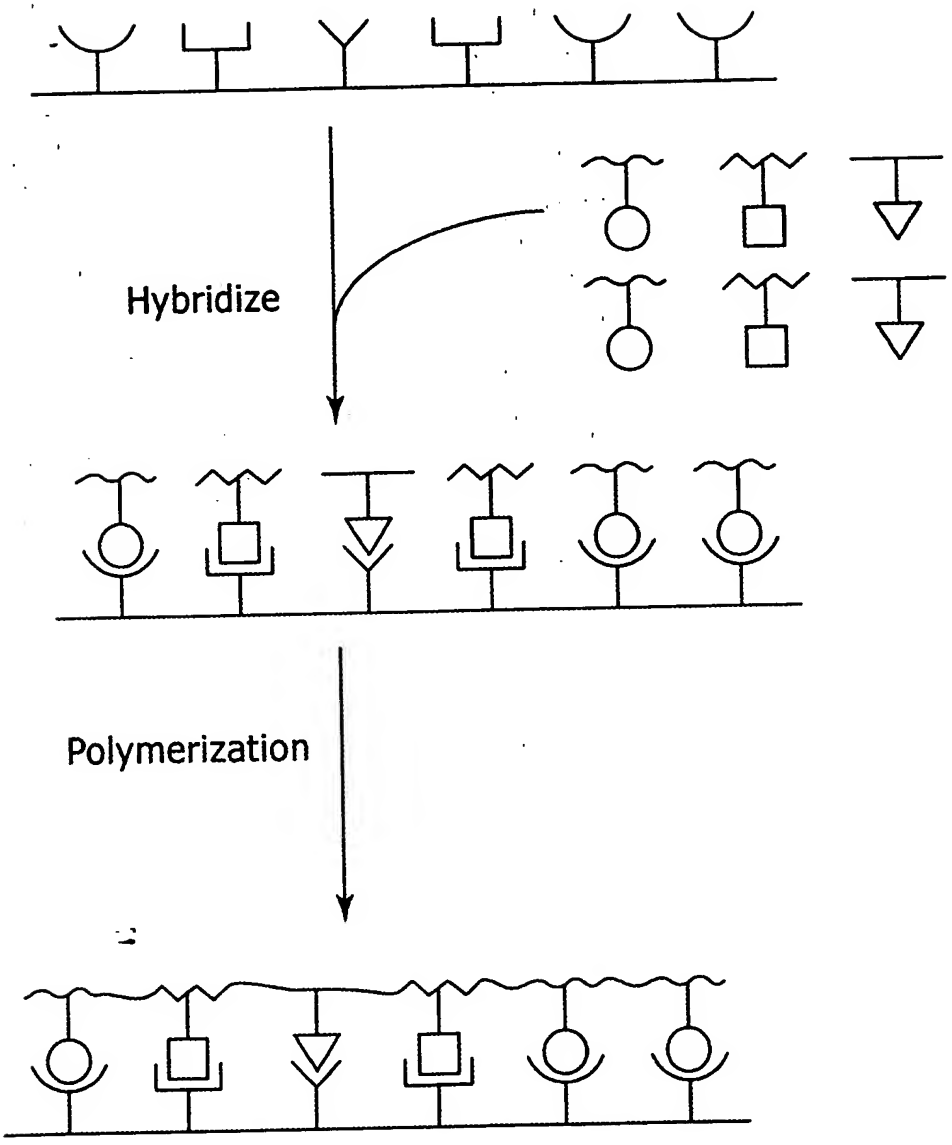

X = H, OH
(L. Orgel)
[H]
(D. Lynn)
X = S, Se
Y = OTs, I
(R. Letsinger, E. Kool)
(G. Joyce, S. Kent)
(G. von Kiedrowski)
(P. Nielsen, L. Orgel)


3'
5'
anticodon
reactive unit
5'
linker
codon
3'
Template
reaction product
3'
5'
5'
codon

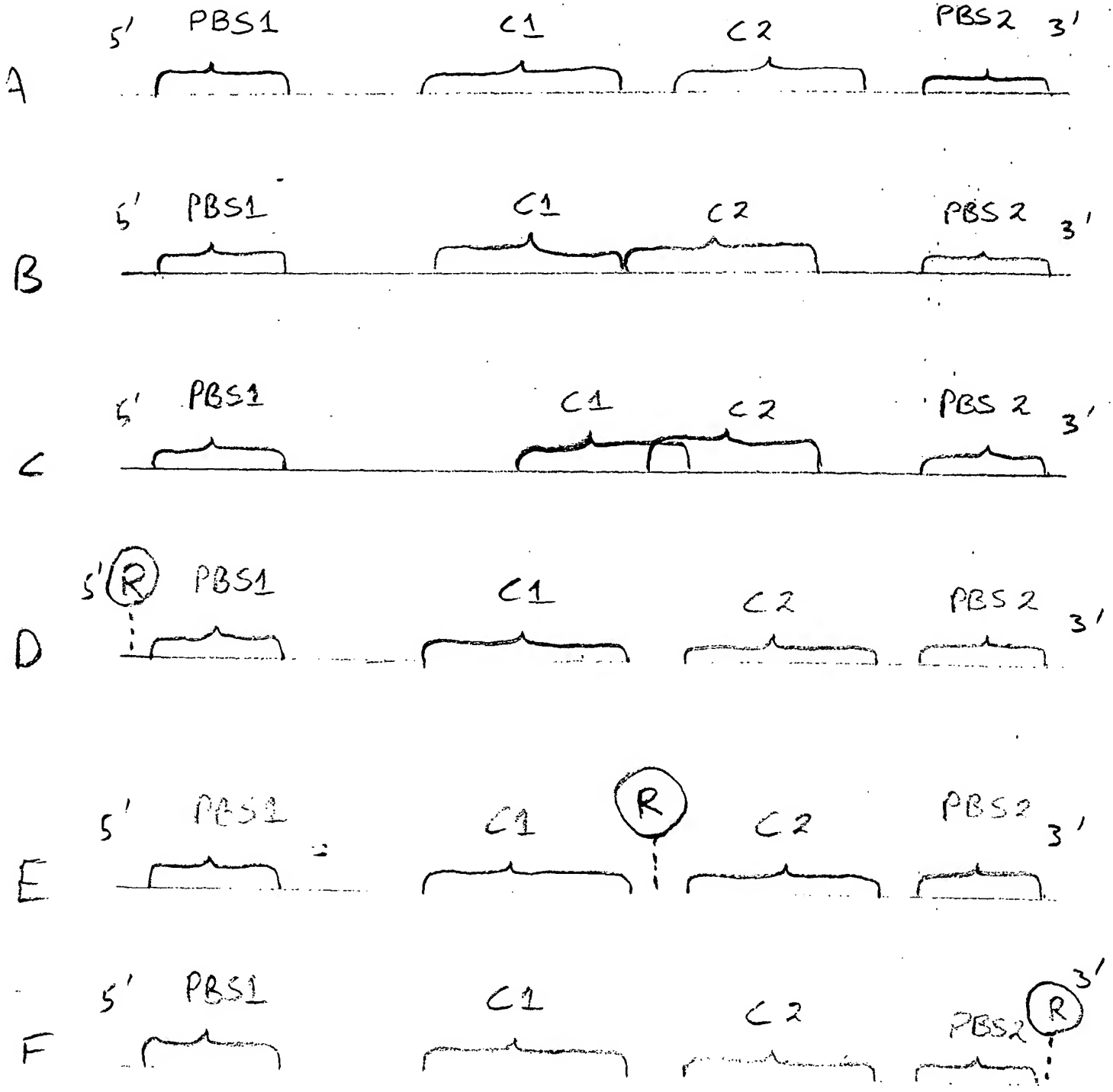
2

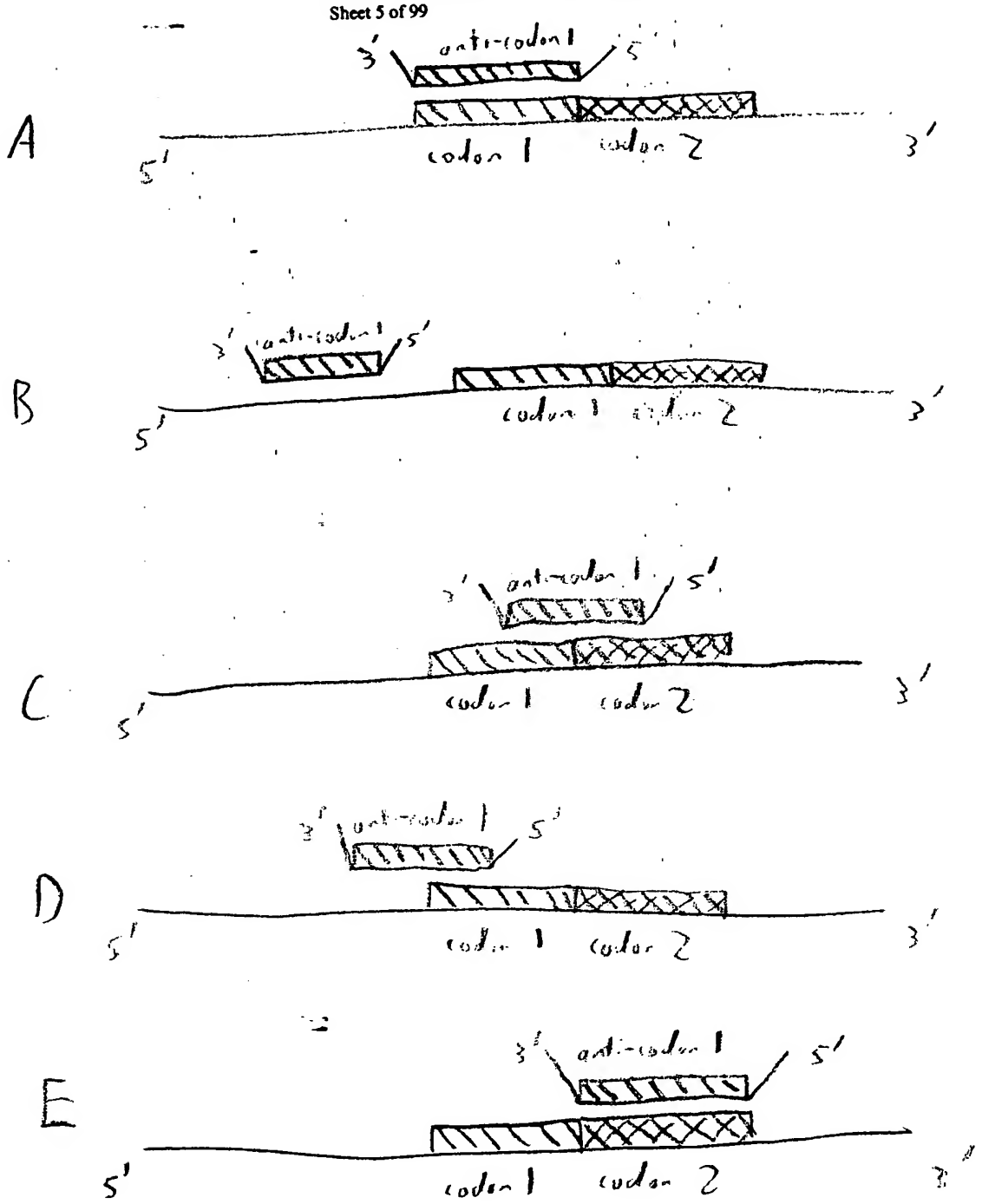

3'
5'
anticodon 2
5'
5'
anticodon 1
5'
codon 1
codon 2
3'
reaction product
3'
5'
3'
5'
5'
codon 1
3'

3



4

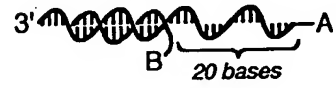

A
5'
PBS1
C1
C2
PBS2
3'
B
5'
PBS1
C1
C2
PBS2
3'
C
5'
PBS1
C1
C2
PBS2
3'
D
5'
R
PBS1
C1
C2
PBS2
3'
E
5'
PBS1
C1
R
C2
PBS2
3'
F
5'
PBS1
C1
C2
PBS2
R
3'


A
3'
anti-codon 1
5'
5'
codon 1
codon 2
3'
B
3'
anti-codon 1
5'
5'
codon 1
codon 2
3'
C
3'
anti-codon 1
5'
5'
codon 1
codon 2
3'
D
3'
anti-codon 1
5'
5'
codon 1
codon 2
3'
E
3'
anti-codon 1
5'
5'
codon 1
codon 2
3'

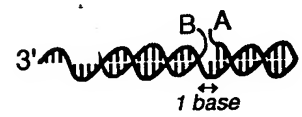
A *end-of-helix (E) n=1*

B *end-of-helix (E) n=10*

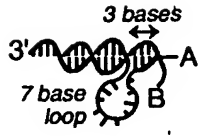
C *end-of-helix (E) n=20*



D *hairpin (H) n=1*

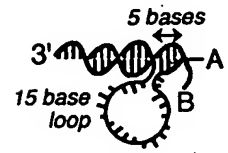


omega, 3-base constant region (Ω-3) n=10



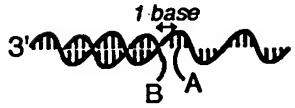
E

omega, 5-base constant region (Ω-5) n=20

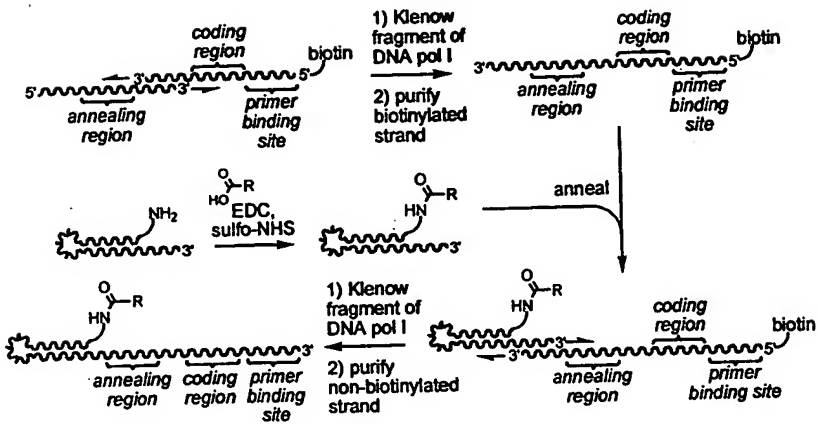


F

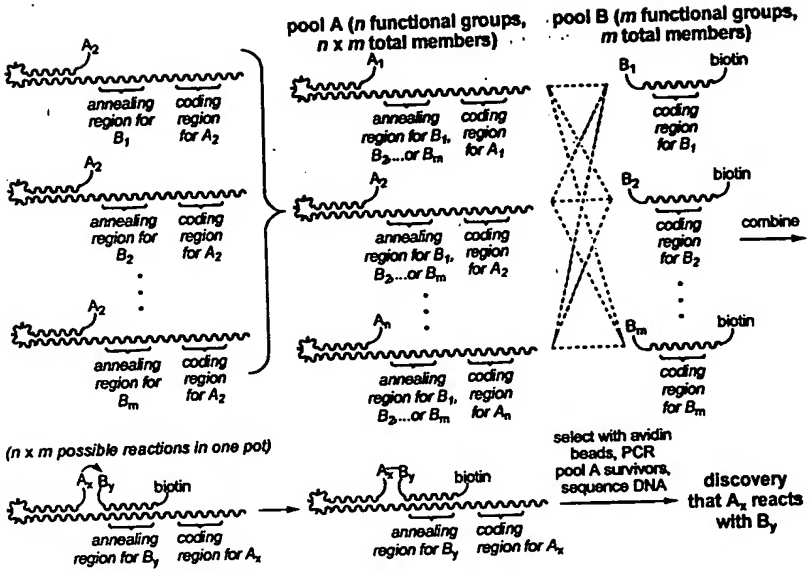
T architecture (T) n=1

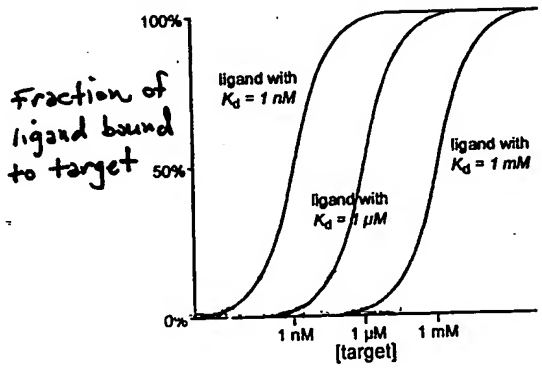


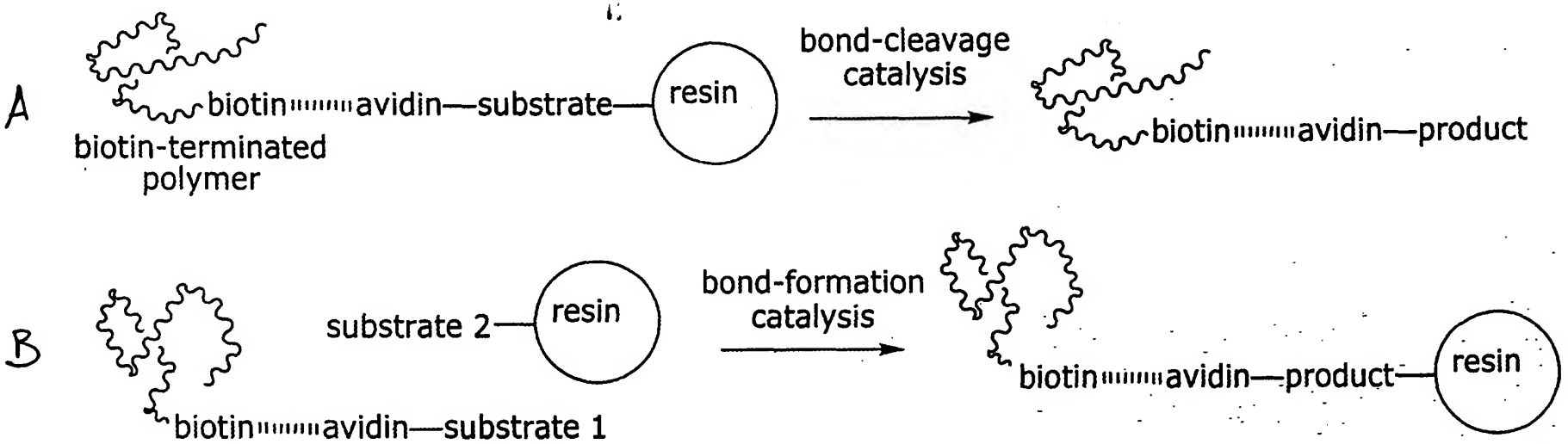
G

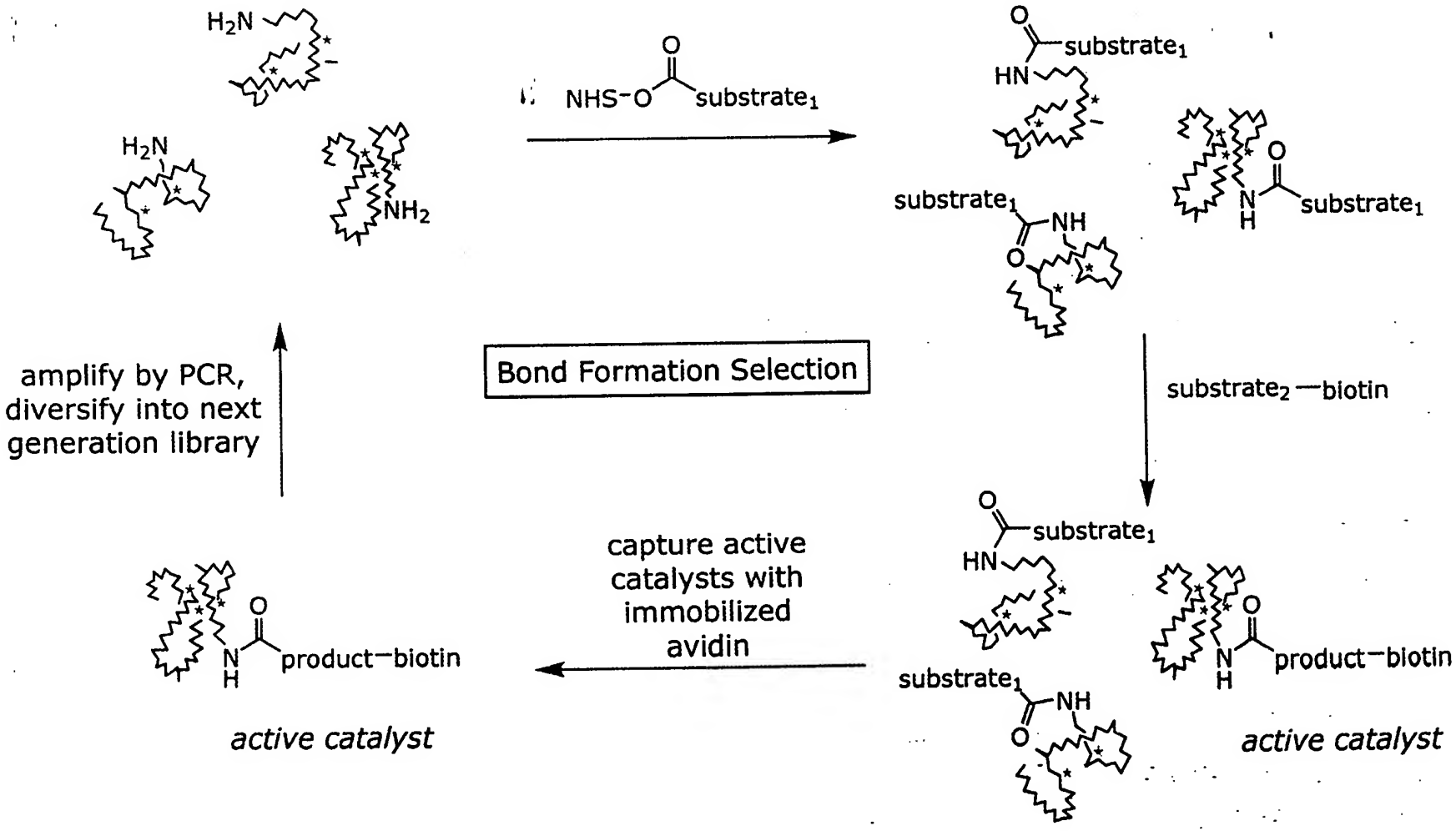

coding region
biotin
5'
3'
3'
5'
annealing region
primer binding site
1) Klenow fragment of DNA pol I
2) purify biotinylated strand
coding region
biotin
3'
5'
annealing region
primer binding site
anneal
NH2
3'
HO
R
EDC, sulfo-NHS
HN
R
3'
HN
R
1) Klenow fragment of DNA pol I
2) purify non-biotinylated strand
3'
annealing region
coding region
primer binding site
HN
R
coding region
biotin
3'
5'
annealing region
primer binding site

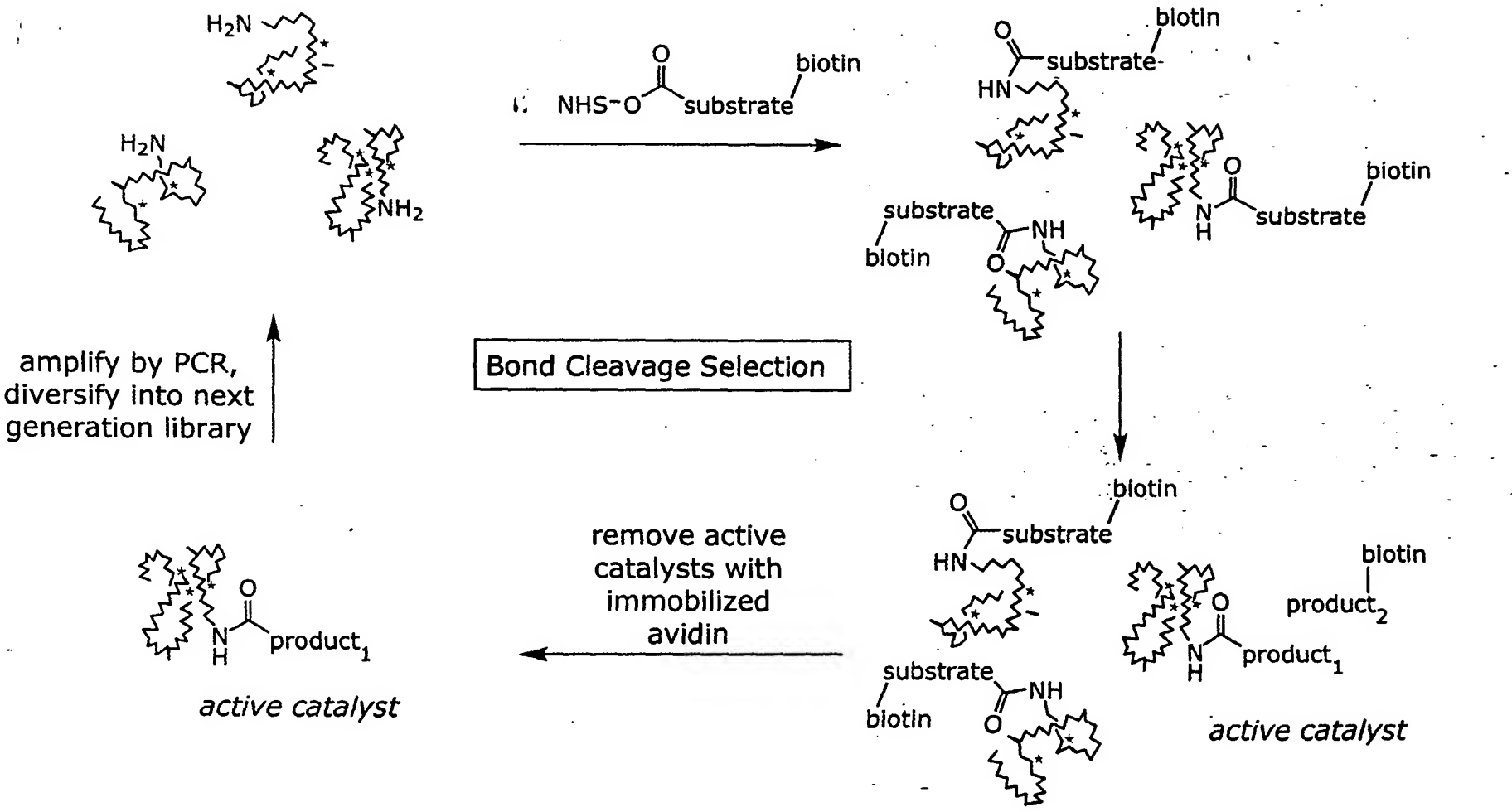
8

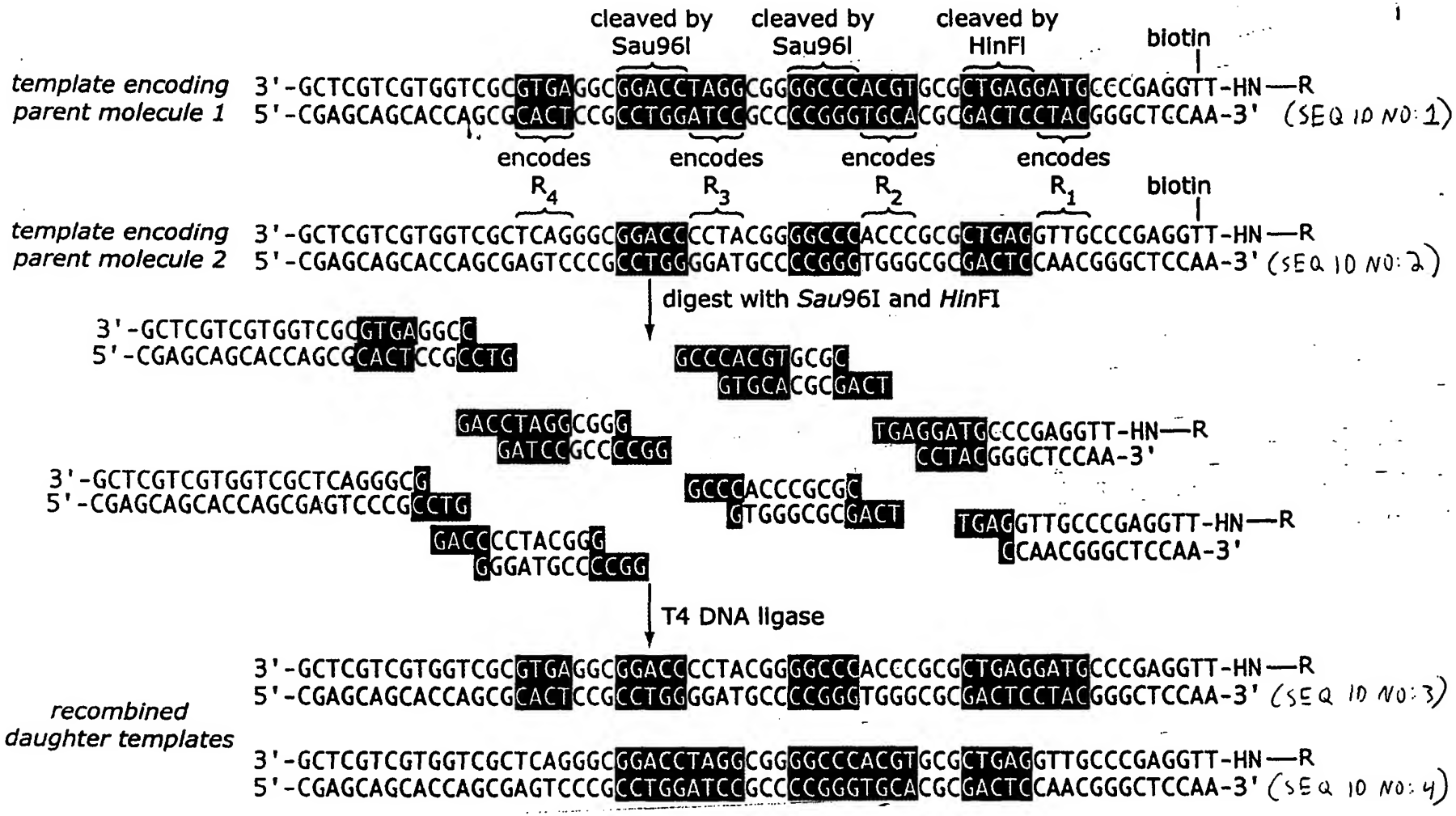

pool A (n functional groups, n x m total members)
pool B (m functional groups, m total members)
annealing region for B1
coding region for A2
annealing region for B2
coding region for A2
annealing region for Bm
coding region for A2
annealing region for B1, B2...or Bm
coding region for A1
annealing region for B1, B2...or Bm
coding region for A2
annealing region for B1, B2...or Bm
coding region for An
biotin
coding region for B1
biotin
coding region for B2
combine
biotin
coding region for Bm
(n x m possible reactions in one pot)
biotin
annealing region for By
coding region for Ax
biotin
annealing region for By
coding region for Ax
select with avidin beads, PCR pool A survivors, sequence DNA
discovery that Ax reacts with By

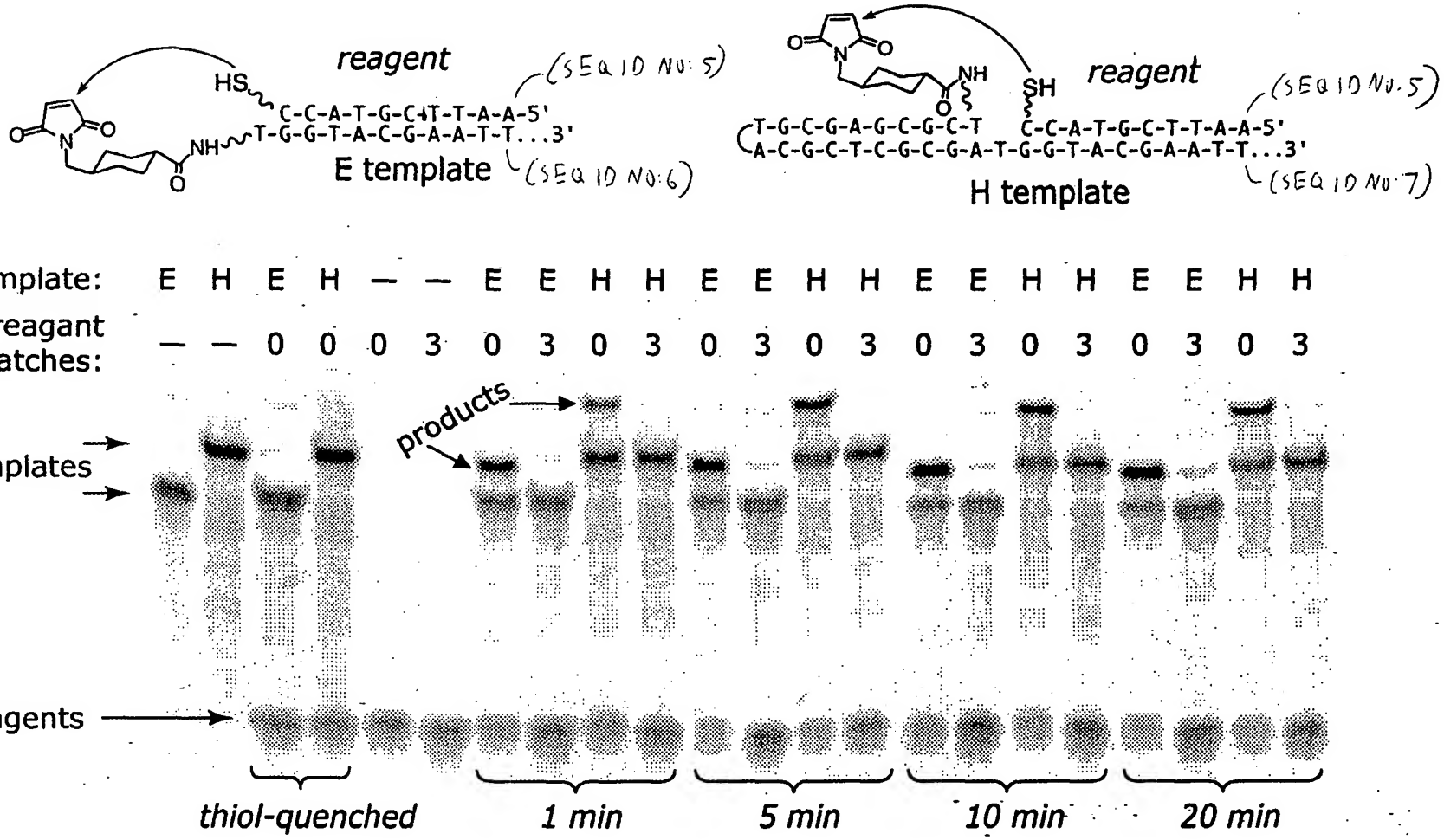

Fraction of ligand bound to target
100%
50%
0%
ligand with K_d = 1 nM
ligand with K_d = 1 μM
ligand with K_d = 1 mM
1 nM
1 μM
1 mM
[target]

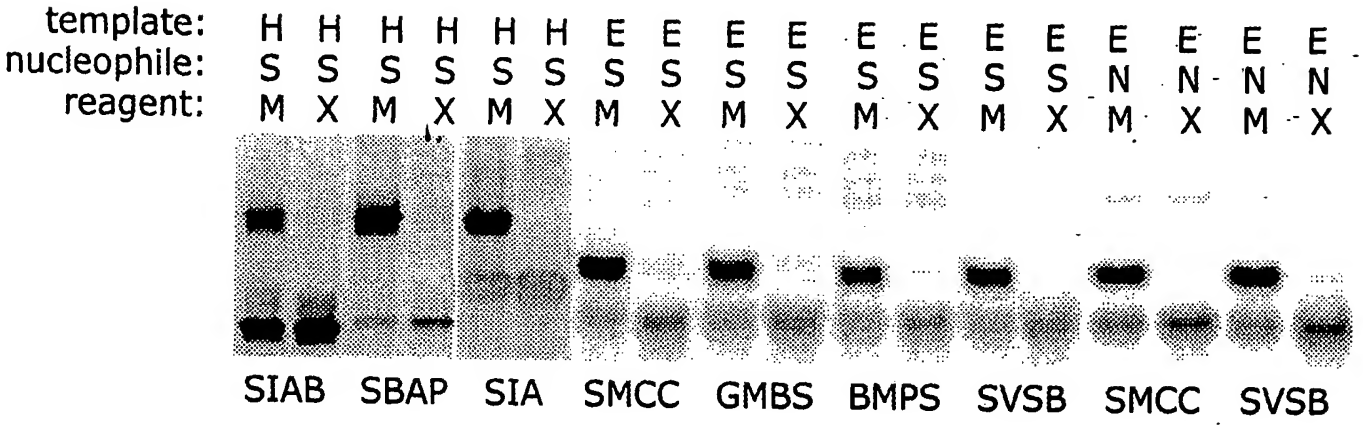

A
biotin-terminated polymer
biotin
avidin
substrate
resin
bond-cleavage catalysis
biotin
avidin—product
B
substrate 2
resin
bond-formation catalysis
biotin
avidin—substrate 1
biotin
avidin—product
resin

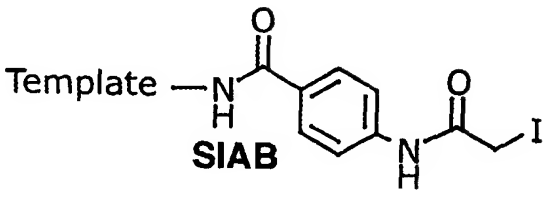

H_2N
H_2N
NH_2
NHS–O
substrate$_1$
substrate$_1$
HN
substrate$_1$
NH
O
N
H
substrate$_1$
substrate$_2$—biotin
Bond Formation Selection
amplify by PCR, diversify into next generation library
capture active catalysts with immobilized avidin
substrate$_1$
HN
substrate$_1$
NH
product—biotin
active catalyst
product—biotin
active catalyst

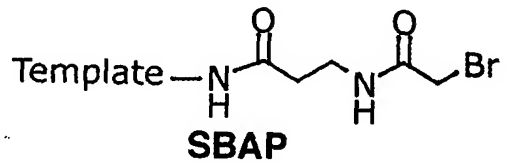

H2N
H2N
NH2
NHS-O
substrate
biotin
biotin
substrate
HN
substrate
biotin
NH
O
biotin
N
H
substrate
Bond Cleavage Selection
amplify by PCR,
diversify into next
generation library
biotin
substrate
HN
biotin
product2
N
H
product1
remove active
catalysts with
immobilized
avidin
substrate
biotin
NH
active catalyst
N
H
product1
active catalyst

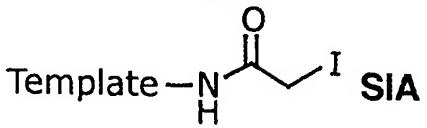

cleaved by Sau96I
cleaved by Sau96I
cleaved by HinFI
biotin
template encoding parent molecule 1
3'-GCTCGTCGTGGTCGCGTGAGGCGGACCTAGGCGGGGCCCACGTGCGCTGAGGATGCCCGAGGTT-HN—R
5'-CGAGCAGCACCAGCGCACTCCGCCTGGATCCGCCCCGGGTGCACGCGACTCCTACGGGCTCCAA-3' (SEQ ID NO:1)
encodes R4
encodes R3
encodes R2
encodes R1
biotin
template encoding parent molecule 2
3'-GCTCGTCGTGGTCGCTCAGGGCGGACCCCTACGGGGCCCACCCGCGCTGAGGTTGCCCGAGGTT-HN—R
5'-CGAGCAGCACCAGCGAGTCCCGCCTGGGGATGCCCCGGGTGGGCGCGACTCCAACGGGCTCCAA-3' (SEQ ID NO:2)
digest with Sau96I and HinFI
3'-GCTCGTCGTGGTCGCGTGAGGCC
5'-CGAGCAGCACCAGCGCACTCCGCCTG
GCCCACGTGCGC
GTGCACGCGACT
GACCTAGGCGGG
GATCCGCCCCGG
TGAGGATGCCCGAGGTT-HN—R
CCTACGGGCTCCAA-3'
3'-GCTCGTCGTGGTCGCTCAGGGCG
5'-CGAGCAGCACCAGCGAGTCCCGCCTG
GCCCACCCGCGC
GTGGGCGCGACT
TGAGGTTGCCCGAGGTT-HN—R
CCAACGGGCTCCAA-3'
GACCCCTACGGG
GGGATGCCCCGG
T4 DNA ligase
recombined daughter templates
3'-GCTCGTCGTGGTCGCGTGAGGCGGACCCCTACGGGGCCCACCCGCGCTGAGGATGCCCGAGGTT-HN—R
5'-CGAGCAGCACCAGCGCACTCCGCCTGGGGATGCCCCGGGTGGGCGCGACTCCTACGGGCTCCAA-3' (SEQ ID NO:3)
3'-GCTCGTCGTGGTCGCTCAGGGCGGACCTAGGCGGGGCCCACGTGCGCTGAGGTTGCCCGAGGTT-HN—R
5'-CGAGCAGCACCAGCGAGTCCCGCCTGGATCCGCCCCGGGTGCACGCGACTCCAACGGGCTCCAA-3' (SEQ ID NO:4)

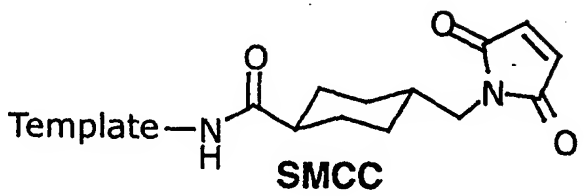

reagent
HS
C-C-A-T-G-C-T-T-A-A-5'
T-G-G-T-A-C-G-A-A-T-T...3'
E template
(SEQ ID NO:5)
(SEQ ID NO:6)
reagent
SH
T-G-C-G-A-G-C-G-C-T
C-C-A-T-G-C-T-T-A-A-5'
A-C-G-C-T-C-G-C-G-A-T-G-G-T-A-C-G-A-A-T-T...3'
H template
(SEQ ID NO:5)
(SEQ ID NO:7)
template: E H E H — — E E H H E E H H E E H H E E H H
# of reagant matches: — — 0 0 0 3 0 3 0 3 0 3 0 3 0 3 0 3 0 3 0 3
products
templates
reagents
thiol-quenched
1 min
5 min
10 min
20 min

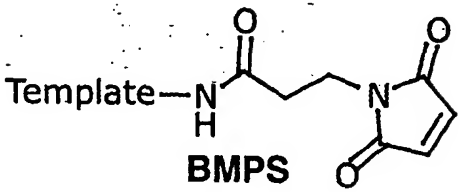

template: H H H H H H E E E E E E E E E E E E
nucleophile: S S S S S S S S S S S S S S N N N N
reagent: M X M X M X M X M X M X M X M X M X
SIAB SBAP SIA SMCC GMBS BMPS SVSB SMCC SVSB

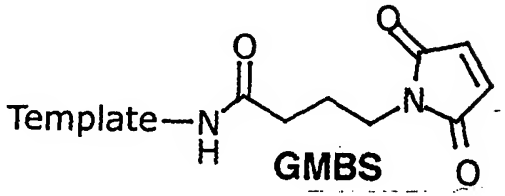

Template
SIAB

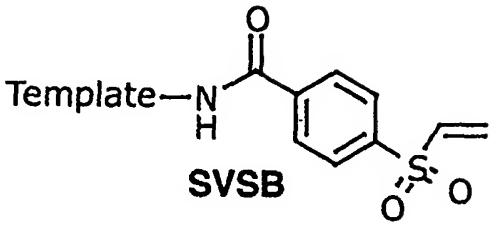

Template
Br
SBAP

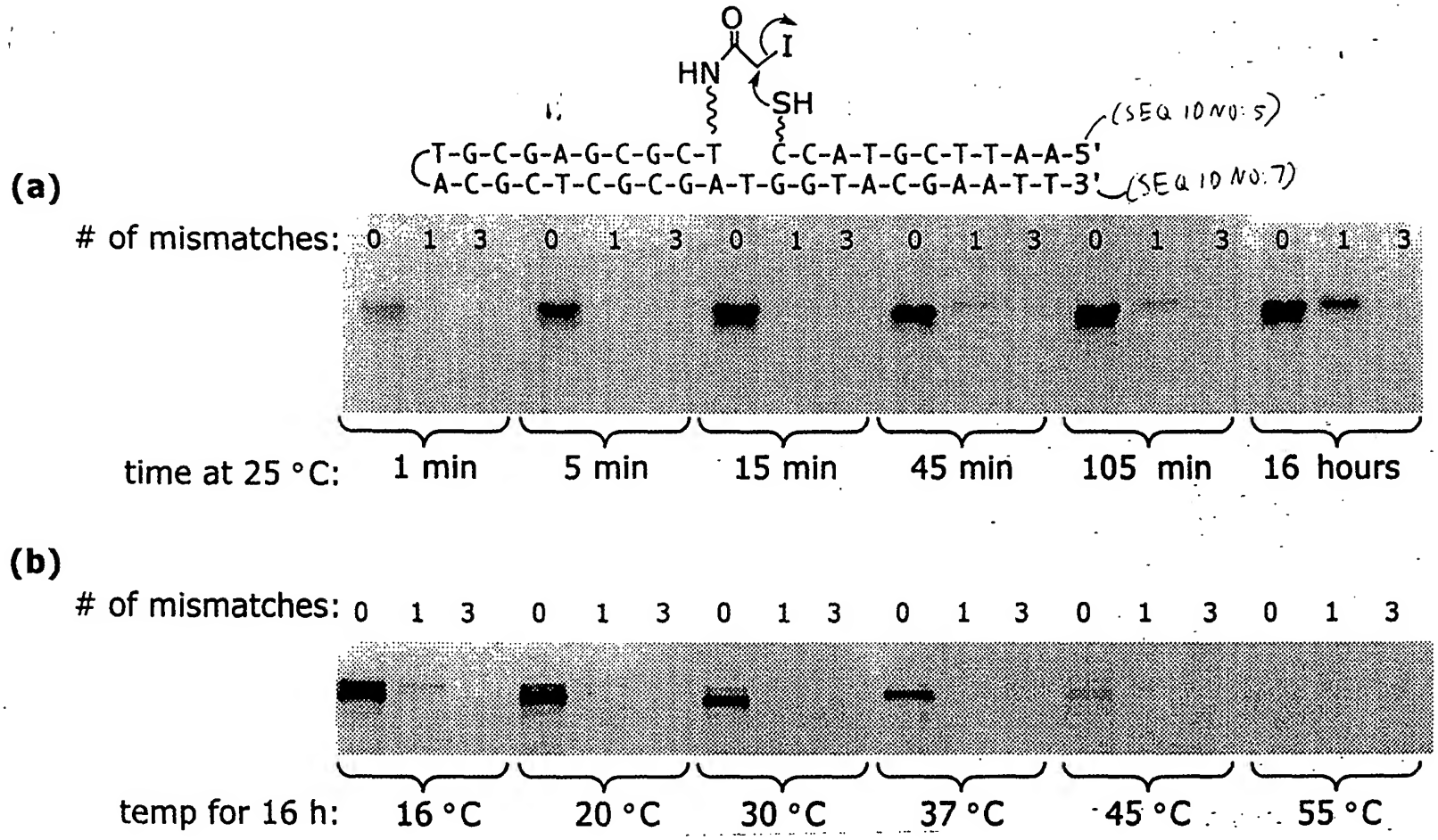

Template
SIA

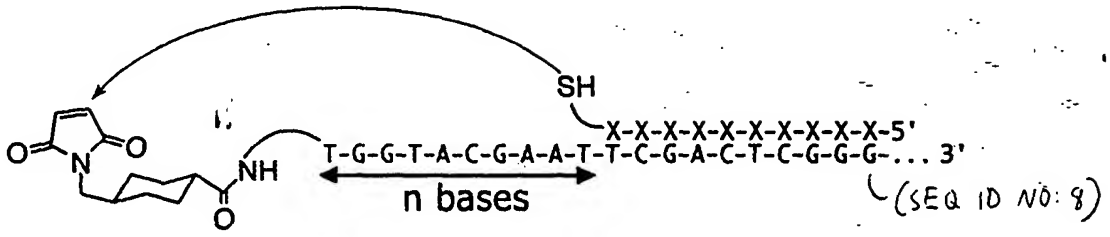

Template
SMCC

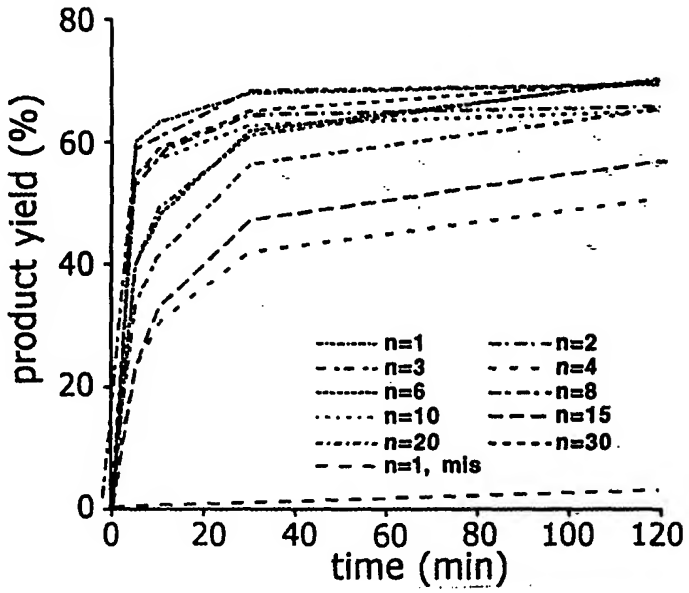

Template
BMPS

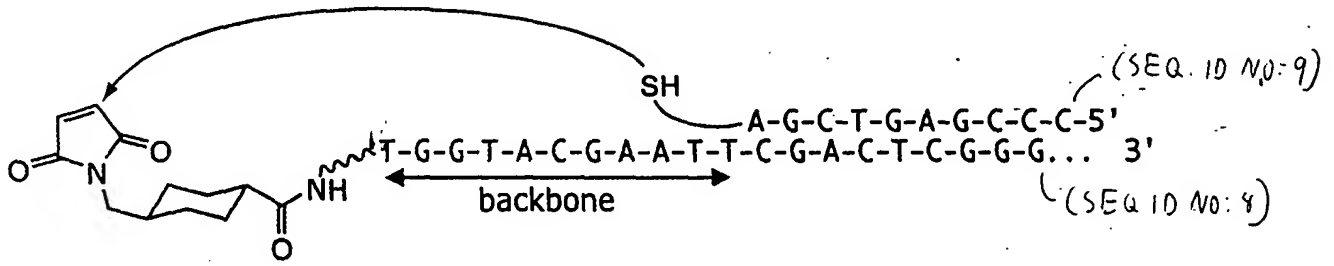

Template
GMBS

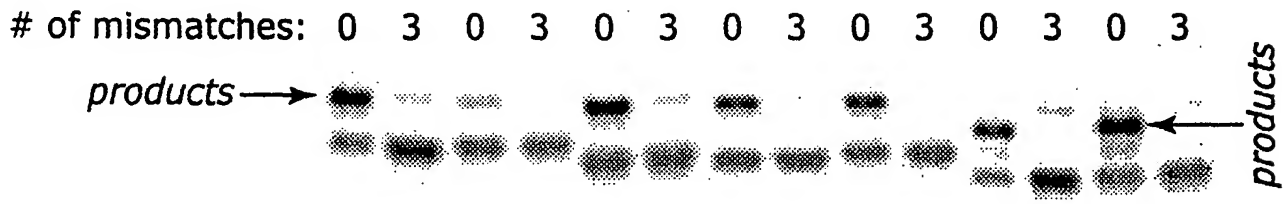

Template
SVSB

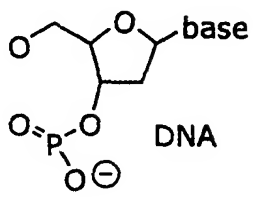

O
I
HN
SH
T-G-C-G-A-G-C-G-C-T C-C-A-T-G-C-T-T-A-A-5'
A-C-G-C-T-C-G-C-G-A-T-G-G-T-A-C-G-A-A-T-T-3'
(SEQ ID NO: 5)
(SEQ ID NO: 7)
(a)
# of mismatches: 0 1 3 0 1 3 0 1 3 0 1 3 0 1 3 0 1 3
time at 25 °C: 1 min 5 min 15 min 45 min 105 min 16 hours
(b)
# of mismatches: 0 1 3 0 1 3 0 1 3 0 1 3 0 1 3 0 1 3
temp for 16 h: 16 °C 20 °C 30 °C 37 °C 45 °C 55 °C

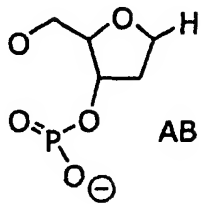

SH
X-X-X-X-X-X-X-X-X-X-5'
T-G-G-T-A-C-G-A-A-T-T-C-G-A-C-T-C-G-G-G-... 3'
NH
n bases
(SEQ ID NO: 8)

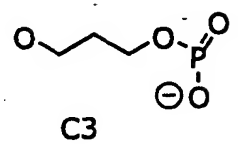

product yield (%)
time (min)
0
20
40
60
80
100
120
n=1
n=2
n=3
n=4
n=6
n=8
n=10
n=15
n=20
n=30
n=1, mis

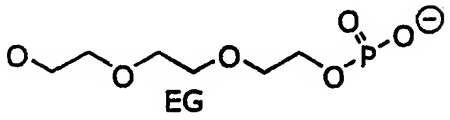

SH
A-G-C-T-G-A-G-C-C-C-5'
(SEQ. ID NO: 9)
T-G-G-T-A-C-G-A-A-T-T-C-G-A-C-T-C-G-G-G... 3'
backbone
(SEQ ID NO: 4)

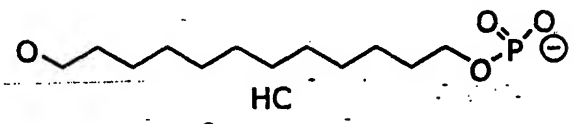

# of mismatches: 0 3 0 3 0 3 0 3 0 3 0 3 0 3
products
products
backbone: (DNA)9 (DNA)9 +clamp (AB)9 (C3)9 (EG)6 (HC)5 (HC)6

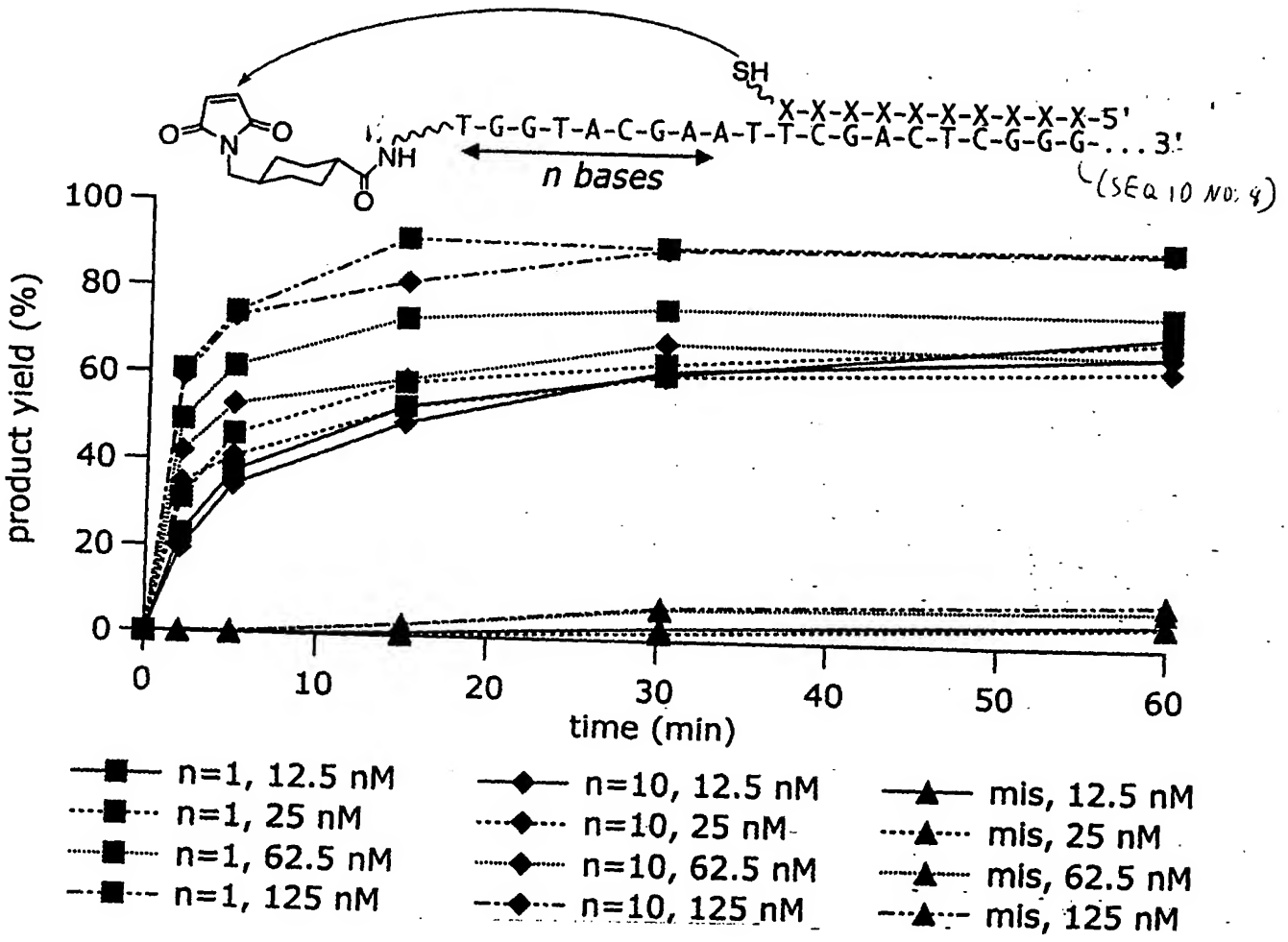

base
DNA

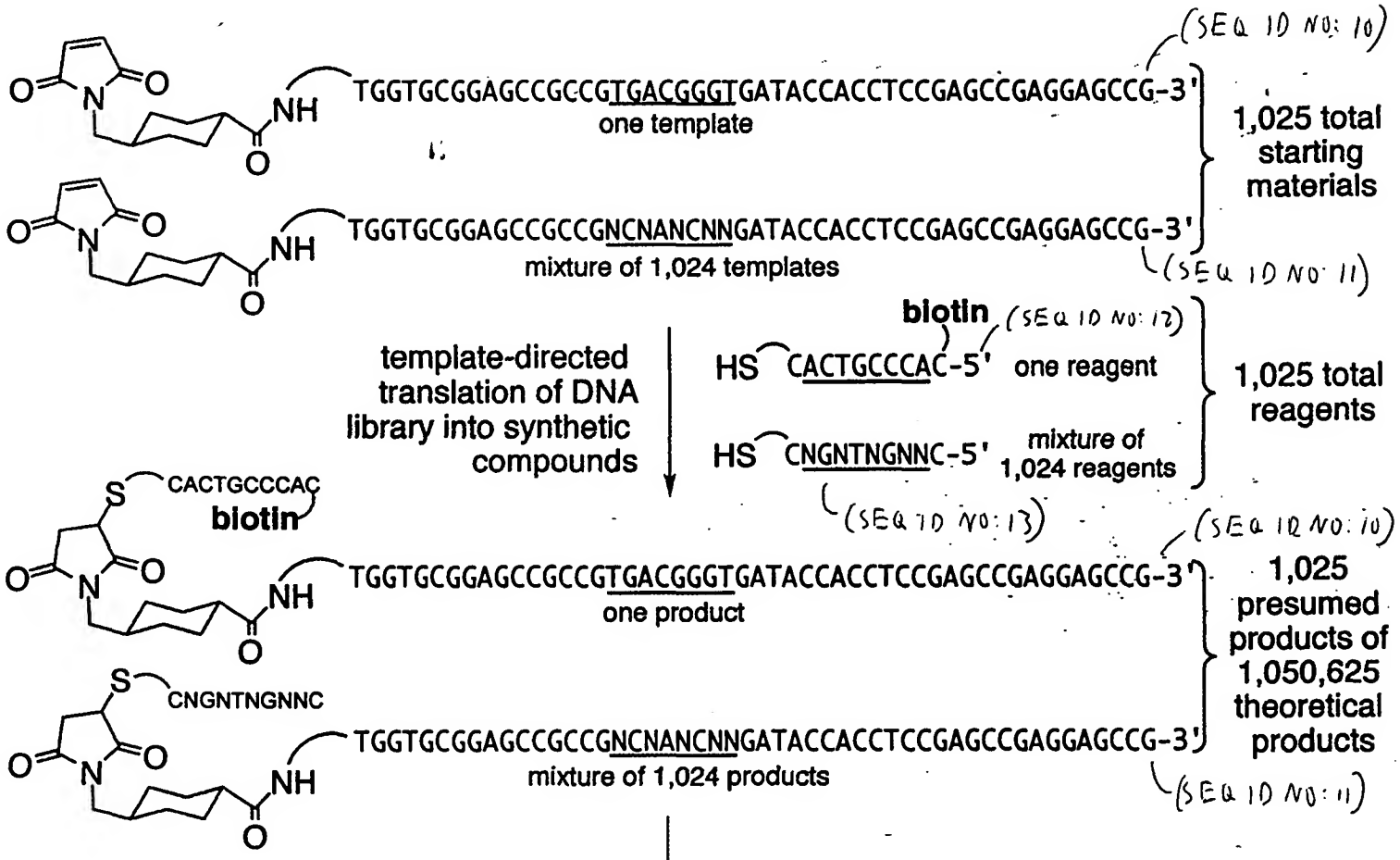

H
AB

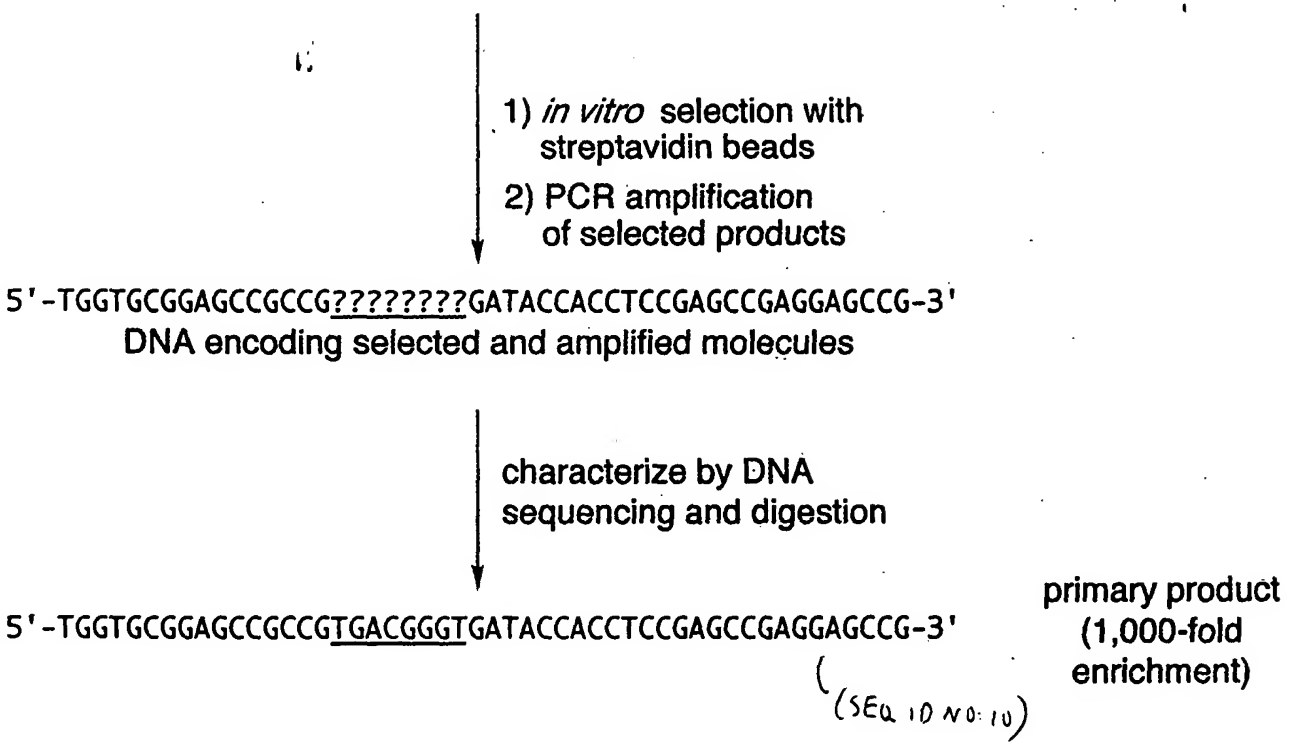

C3


EG

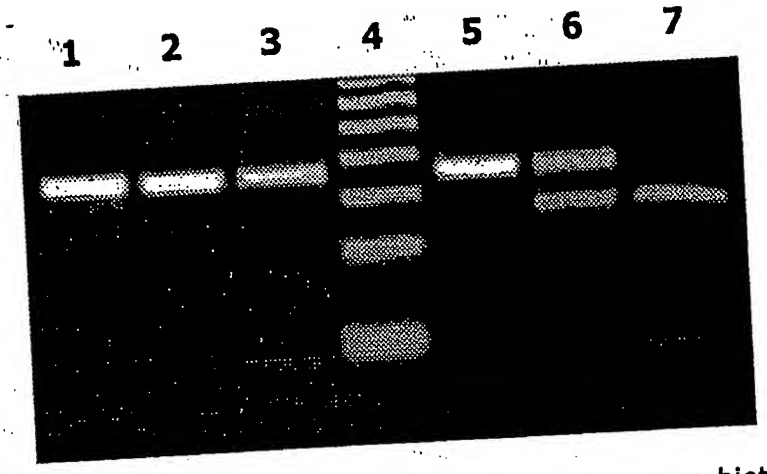

HC


SH
X-X-X-X-X-X-X-X-X-X-5'
T-G-G-T-A-C-G-A-A-T-T-C-G-A-C-T-C-G-G-G-...3'
NH
n bases
(SEQ ID NO: 4)
product yield (%)
100
80
60
40
20
0
0
10
20
30
40
50
60
time (min)
n=1, 12.5 nM
n=1, 25 nM
n=1, 62.5 nM
n=1, 125 nM
n=10, 12.5 nM
n=10, 25 nM
n=10, 62.5 nM
n=10, 125 nM
mis, 12.5 nM
mis, 25 nM
mis, 62.5 nM
mis, 125 nM

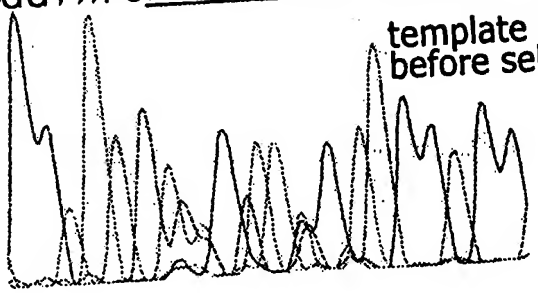

TGGTGCGGAGCCGCCGTGACGGGTGATACCACCTCCGAGCCGAGGAGCCG-3'
one template
(SEQ ID NO: 10)
TGGTGCGGAGCCGCCGNCNANCNNGATACCACCTCCGAGCCGAGGAGCCG-3'
mixture of 1,024 templates
(SEQ ID NO: 11)
1,025 total starting materials
biotin
(SEQ ID NO: 12)
HS CACTGCCCAC-5' one reagent
HS CNGNTNGNNC-5' mixture of 1,024 reagents
(SEQ ID NO: 13)
1,025 total reagents
template-directed translation of DNA library into synthetic compounds
CACTGCCCAC
biotin
CNGNTNGNNC
TGGTGCGGAGCCGCCGTGACGGGTGATACCACCTCCGAGCCGAGGAGCCG-3'
one product
(SEQ ID NO: 10)
TGGTGCGGAGCCGCCGNCNANCNNGATACCACCTCCGAGCCGAGGAGCCG-3'
mixture of 1,024 products
(SEQ ID NO: 11)
1,025 presumed products of 1,050,625 theoretical products


1) *in vitro* selection with streptavidin beads
2) PCR amplification of selected products
5'-TGGTGCGGAGCCGCCG????????GATACCACCTCCGAGCCGAGGAGCCG-3'
DNA encoding selected and amplified molecules
characterize by DNA sequencing and digestion
5'-TGGTGCGGAGCCGCCGTGACGGGTGATACCACCTCCGAGCCGAGGAGCCG-3'
(SEQ ID NO: 10)
primary product (1,000-fold enrichment)

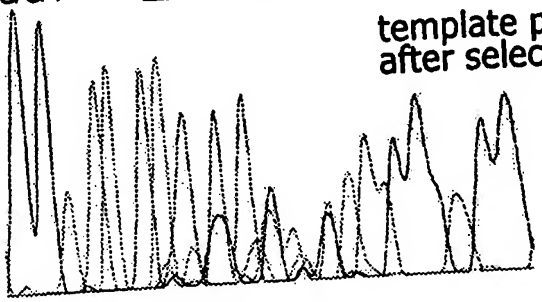
21B

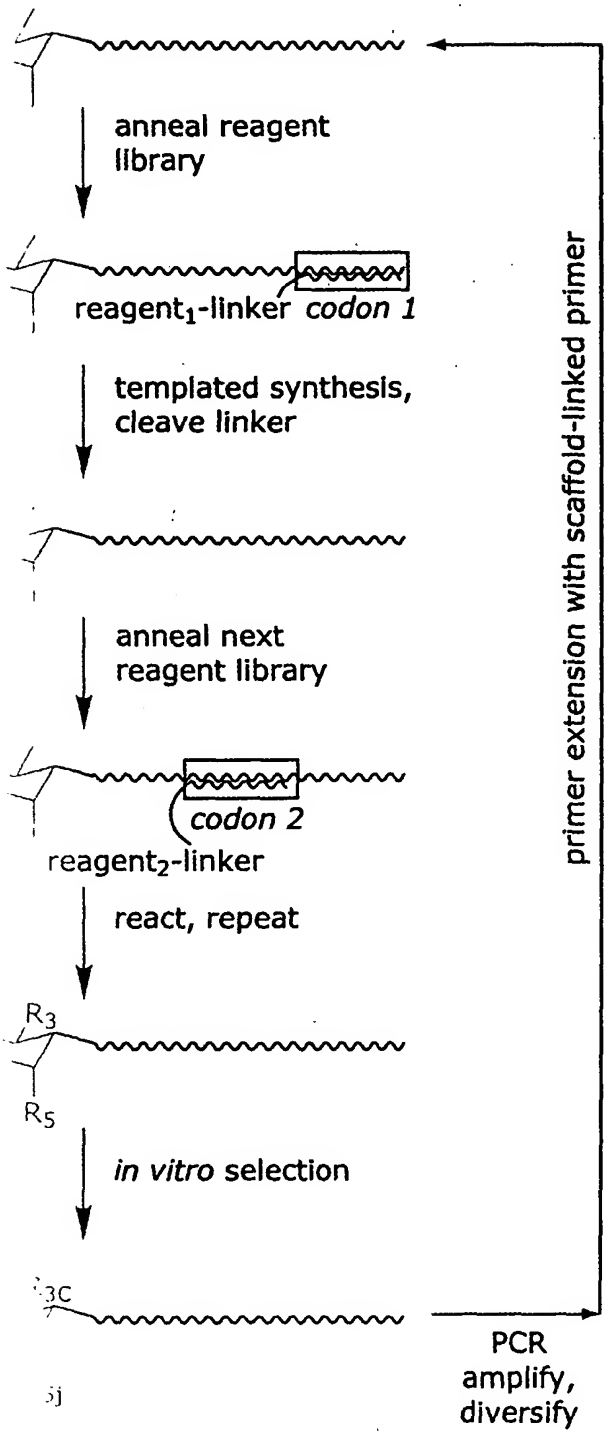


3'---GGT AT CNN G NT NGN CGGCGG--- non-biotin encoding (residues 30-11 of SEQ ID NO: 11)

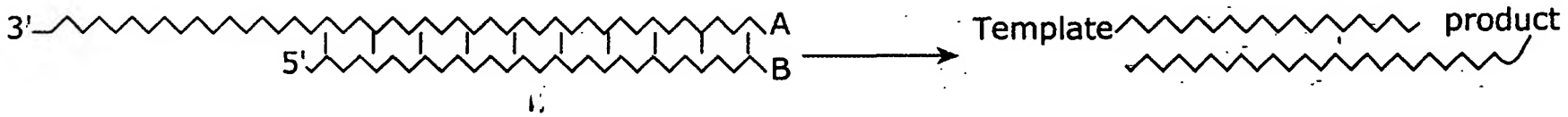


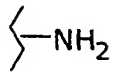
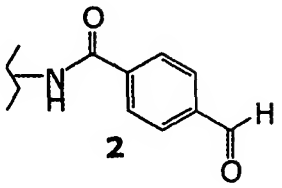
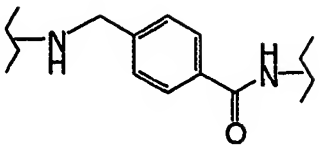
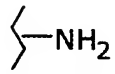
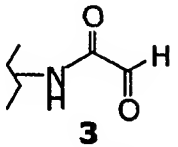
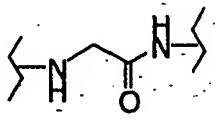
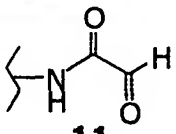
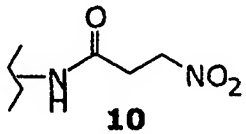
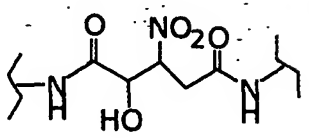
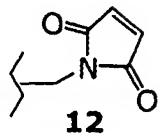
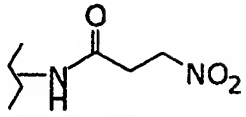
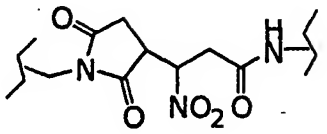
3'---GGT AT CAC C CGT CACGGC GG--- biotin encoding (residues 30-11 of SEQ ID NO: 10)

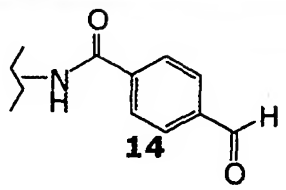
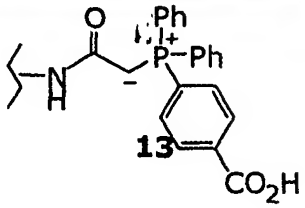
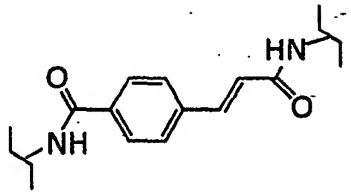
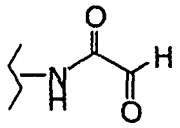
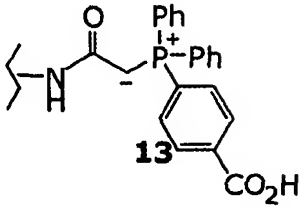
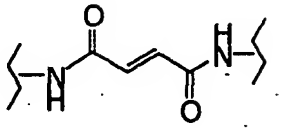
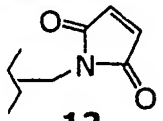
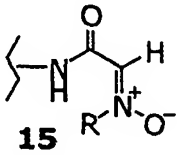
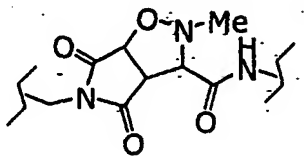
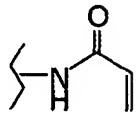
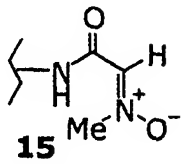
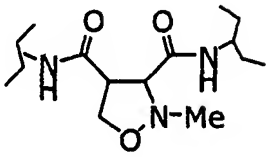



anneal reagent library
reagent$_1$-linker *codon 1*
templated synthesis, cleave linker
anneal next reagent library
codon 2
reagent$_2$-linker
react, repeat
R_3
R_5
in vitro selection
PCR amplify, diversify
primer extension with scaffold-linked primer

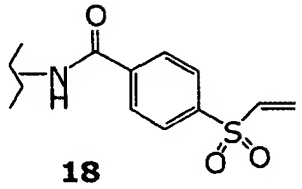
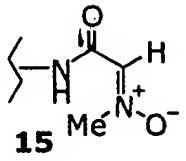
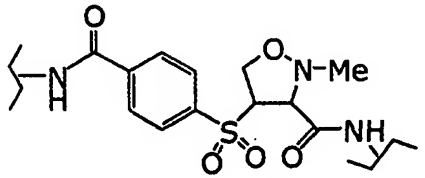
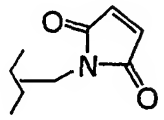
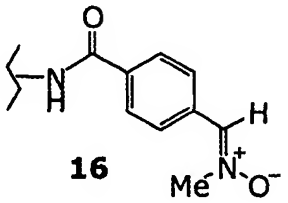
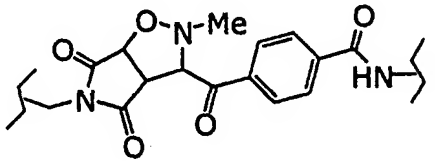
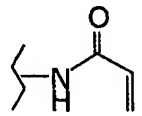
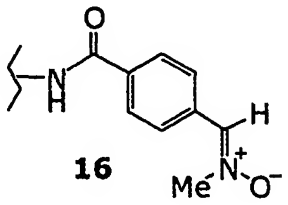
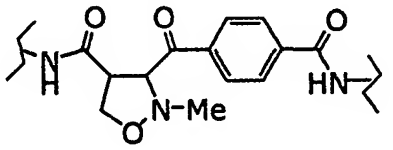
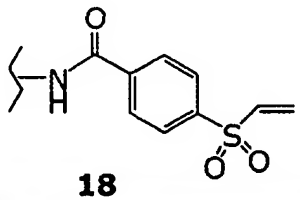
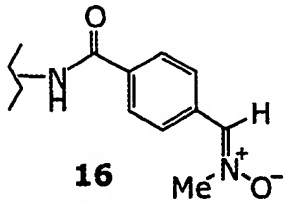
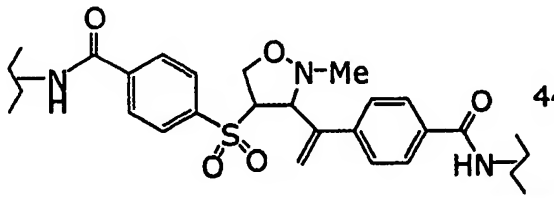
22 B



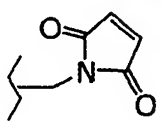
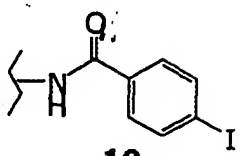
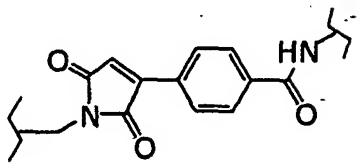
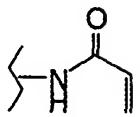
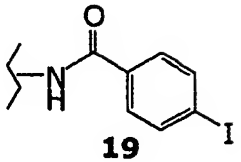
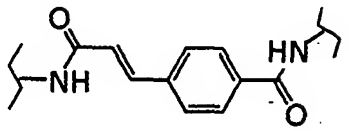
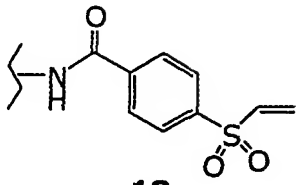
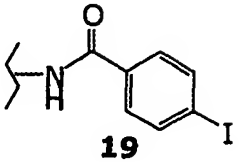
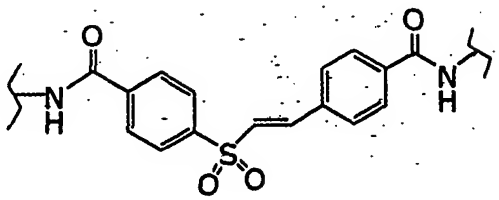
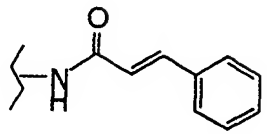
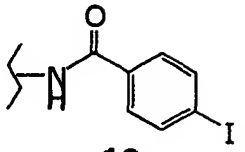
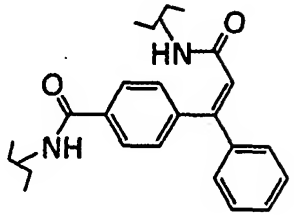
A	B	conditions	product	yield (%)
 **1**	 **2**	a		81
 **1**	 **3**	a		70
 **11**	 **10**	b		45
 **12**	 **10**	b		42

A	B	conditions	product	yield (%)
 **14**	 **13**	c		93
 **11**	 **13**	c		>97
 **12**	 **15**	d		53 (R=Me) 42 (R=Bn)
 **17**	 **15**	d		54

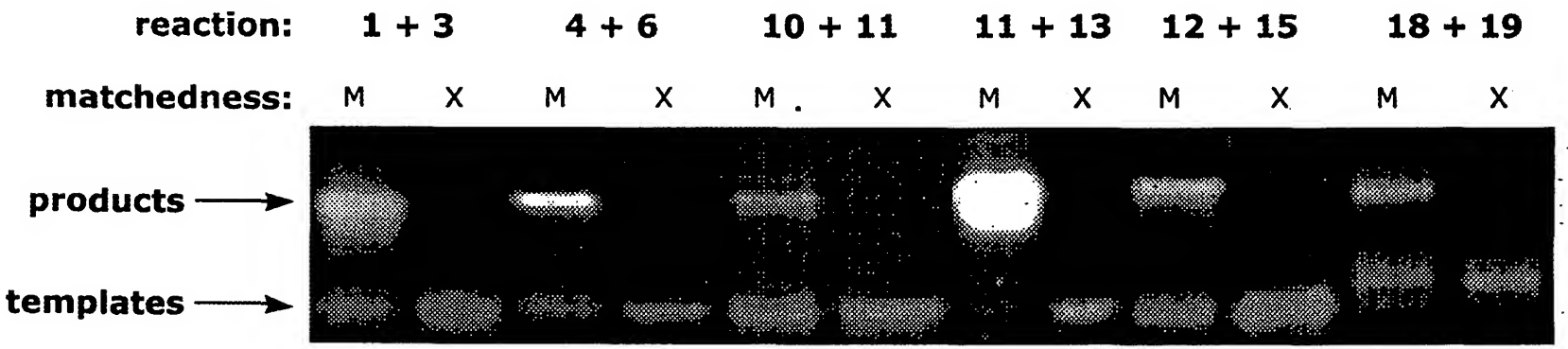
23B

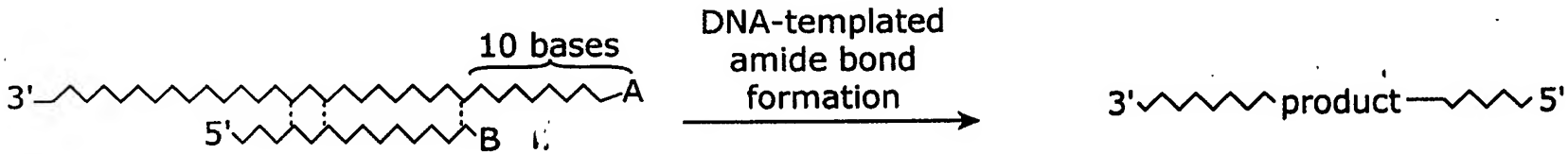
A	B	conditions	product	yield (%)
 **18**	 **15**	d		47
 **12**	 **16**	d		41
 **17**	 **16**	d		15
 **18**	 **16**	d		44

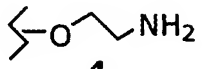
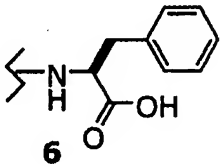
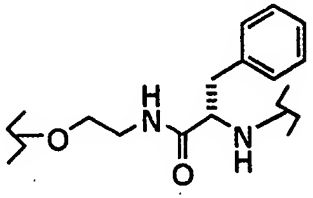
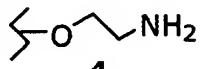
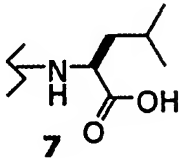
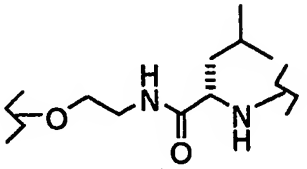
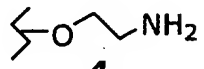
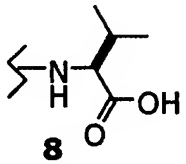
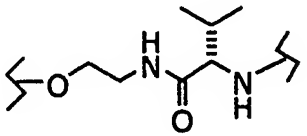
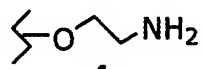
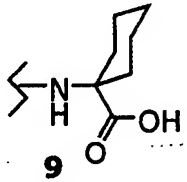
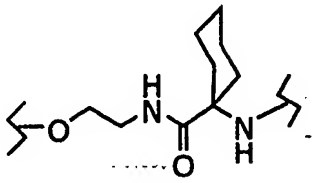
23C

A	B	conditions	product	yield (%)
 **12**	 **19**	e		54
 **17**	 **19**	f		26
 **18**	 **19**	f		51
 **20**	 **19**	f		31

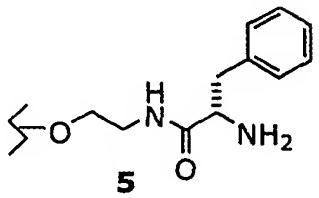
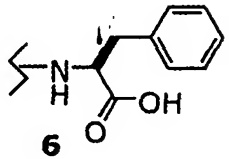
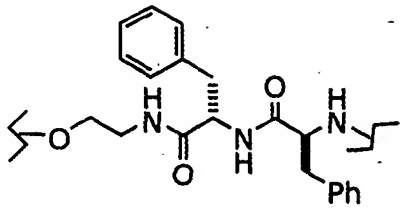
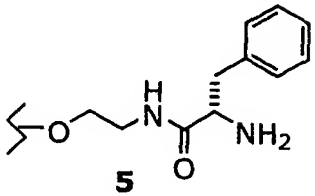
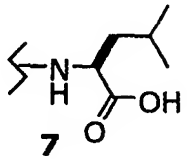
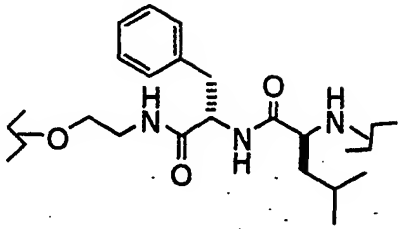
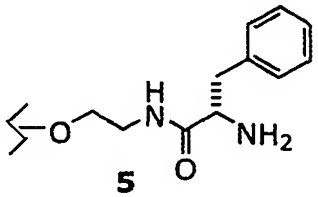
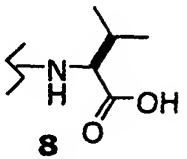
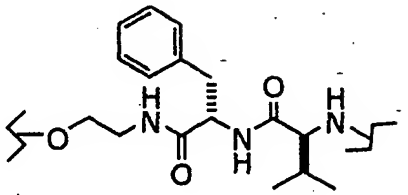
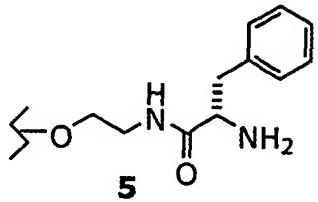
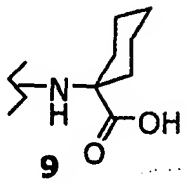
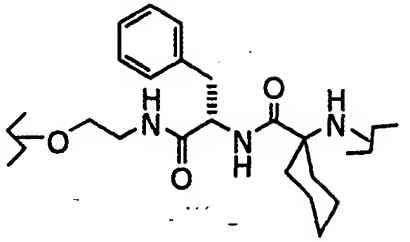
23D


reaction:
1 + 3
4 + 6
10 + 11
11 + 13
12 + 15
18 + 19
matchedness:
M X M X M X M X M X M X
products
templates



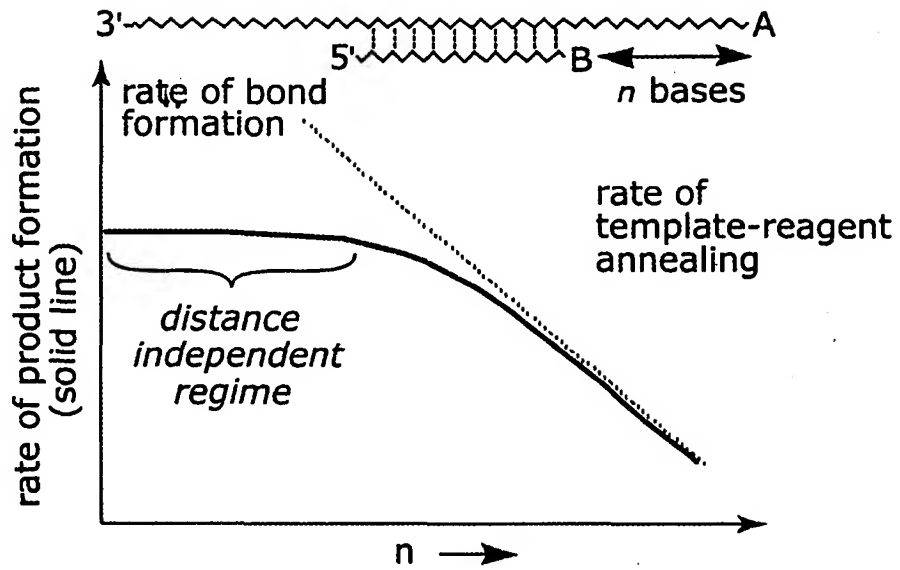
A	B	product	yield (%)
 **4**	 **6**		79, 59
 **4**	 **7**		73, 54
 **4**	 **8**		79, 46
 **4**	 **9**		81, 62

25A

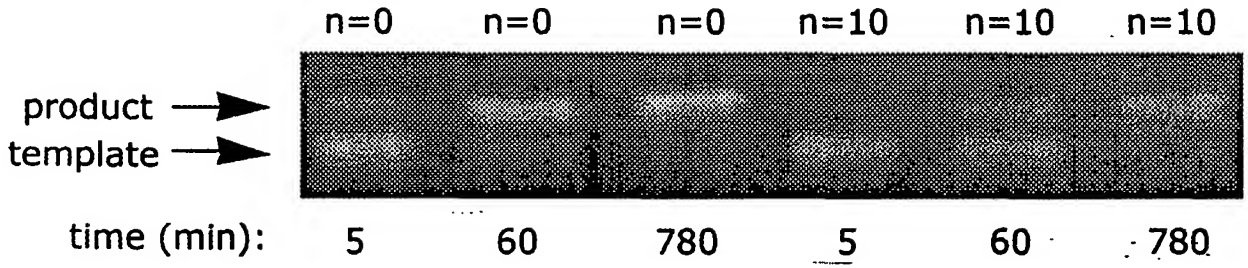
A	B	product	yield (%)
 **5**	 **6**		58, 66
 **5**	 **7**		47, 64
 **5**	 **8**		58, 53
 **5**	 **9**		56, 71

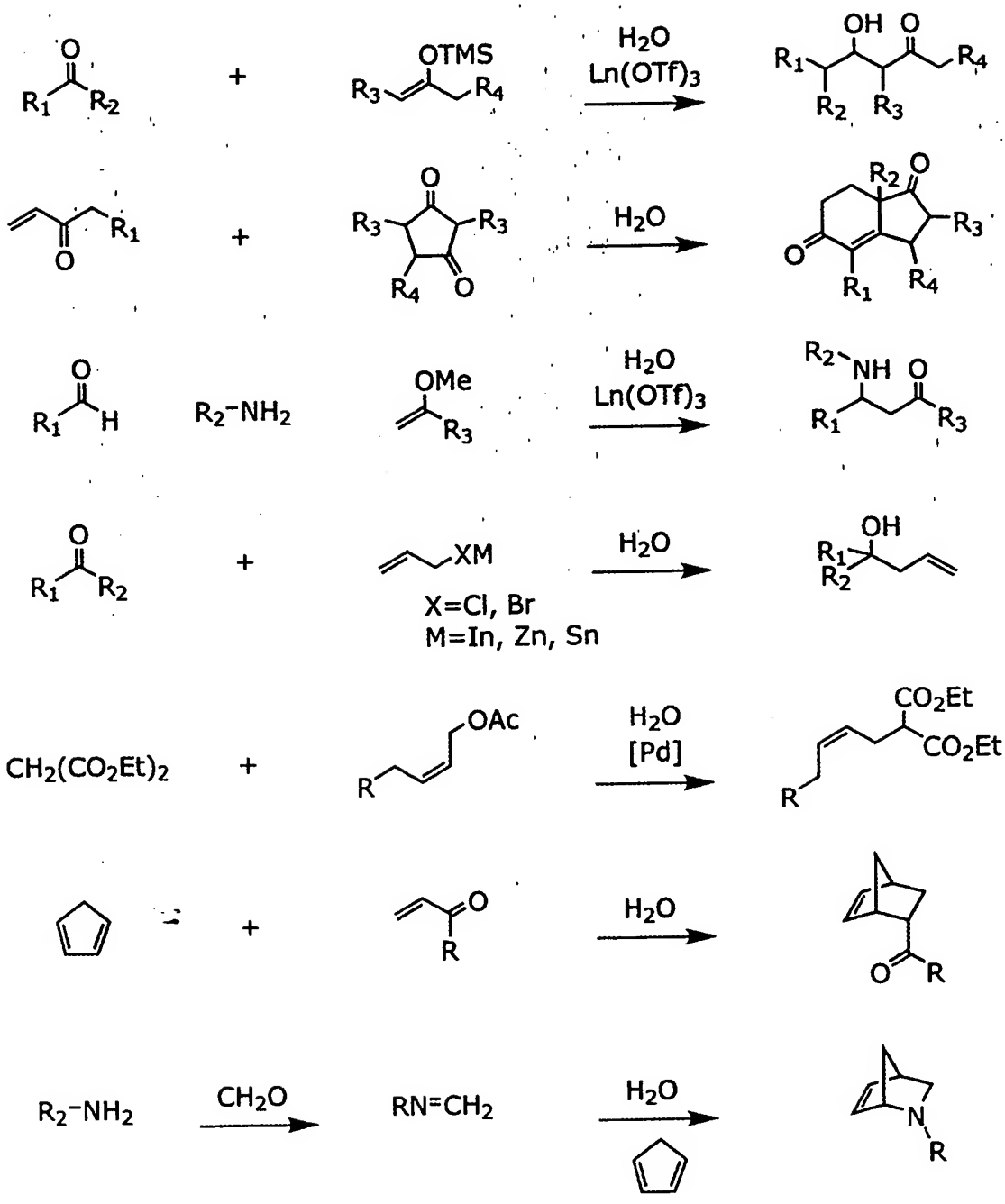
25 B

(a)


3'
A
5'
B
n bases
rate of bond formation
rate of template-reagent annealing
distance independent regime
rate of product formation (solid line)
n

(b)

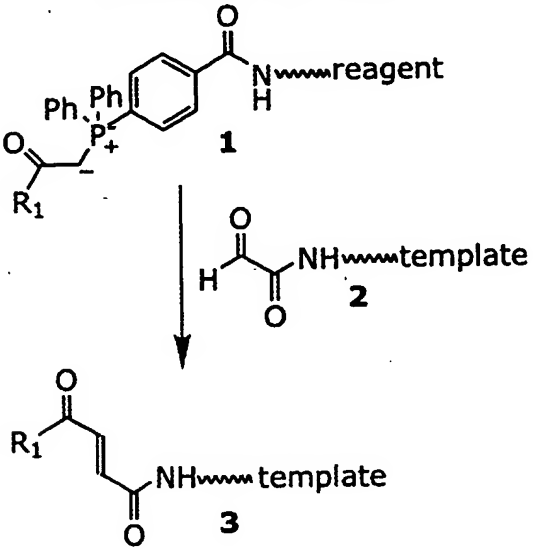

n=0
n=0
n=0
n=10
n=10
n=10
product
template
time (min):
5
60
780
5
60
780


H2O
Ln(OTf)3
OTMS
OH
OMe
XM
X=Cl, Br
M=In, Zn, Sn
CH2(CO2Et)2
OAc
[Pd]
CO2Et
CH2O
RN=CH2

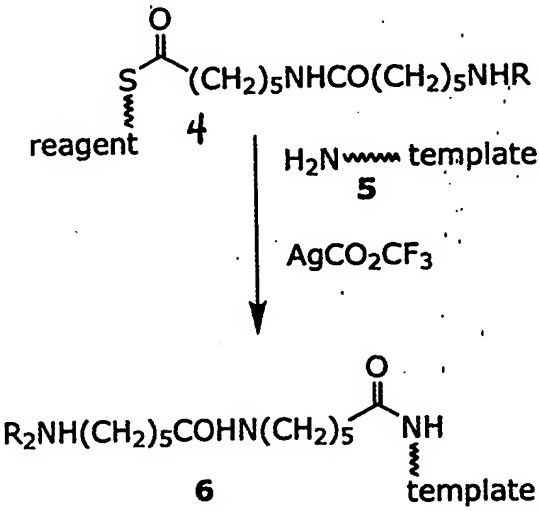
28A

28B

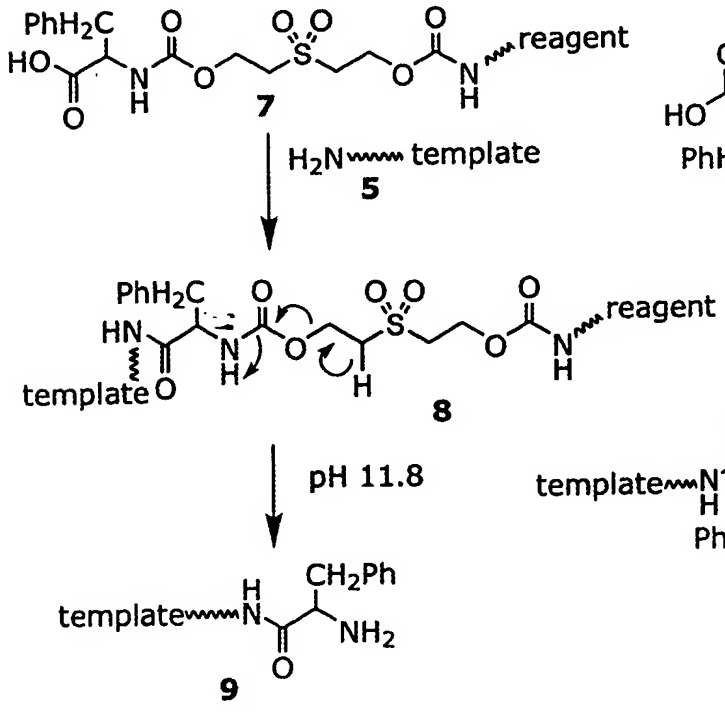
autocleaving linker



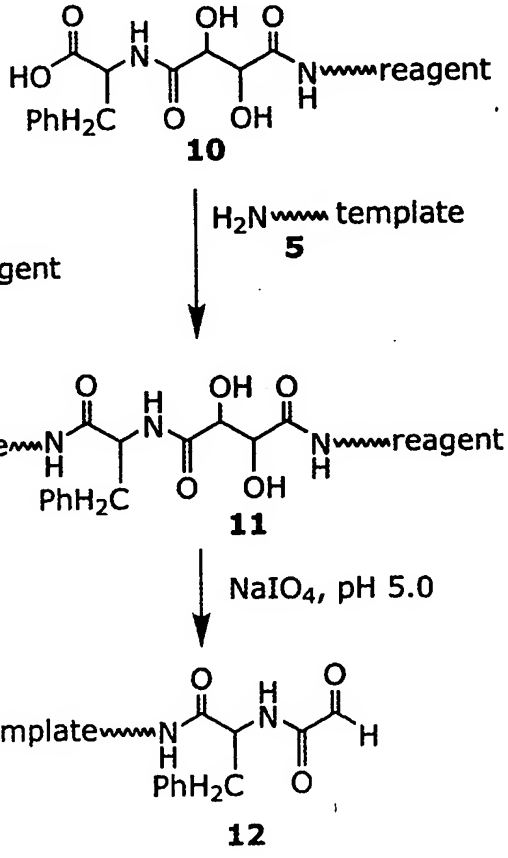
autocleaving linker



scarless linker

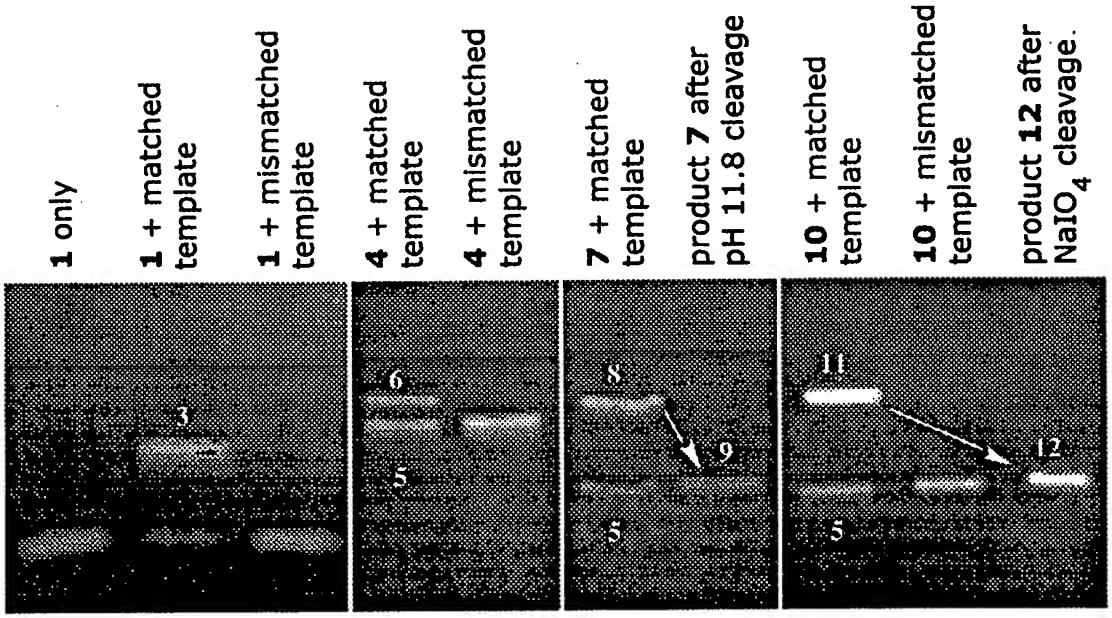


useful scar linker

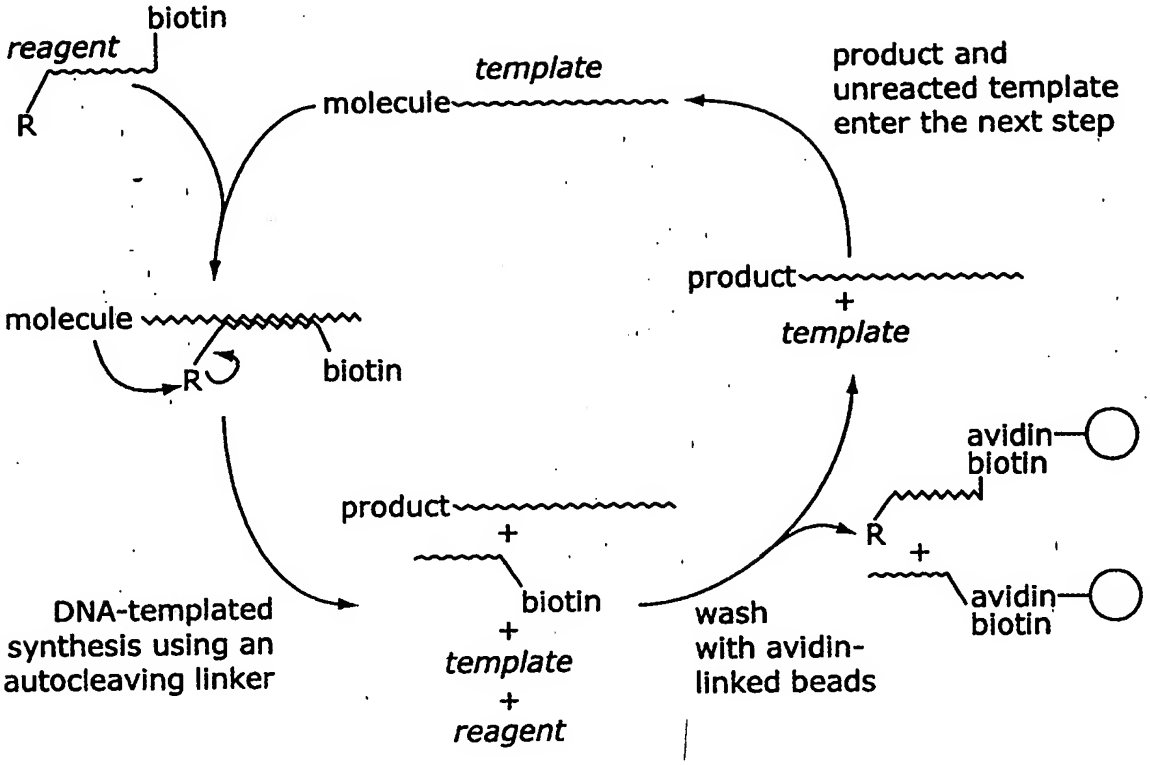


28C

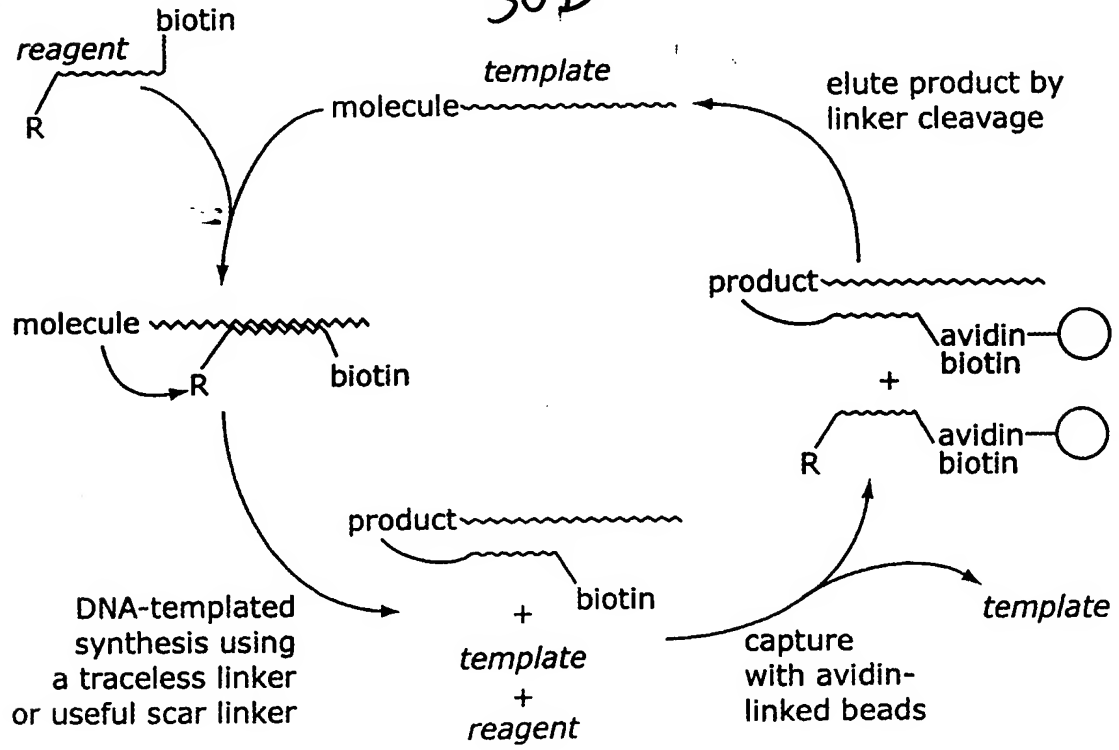
28D

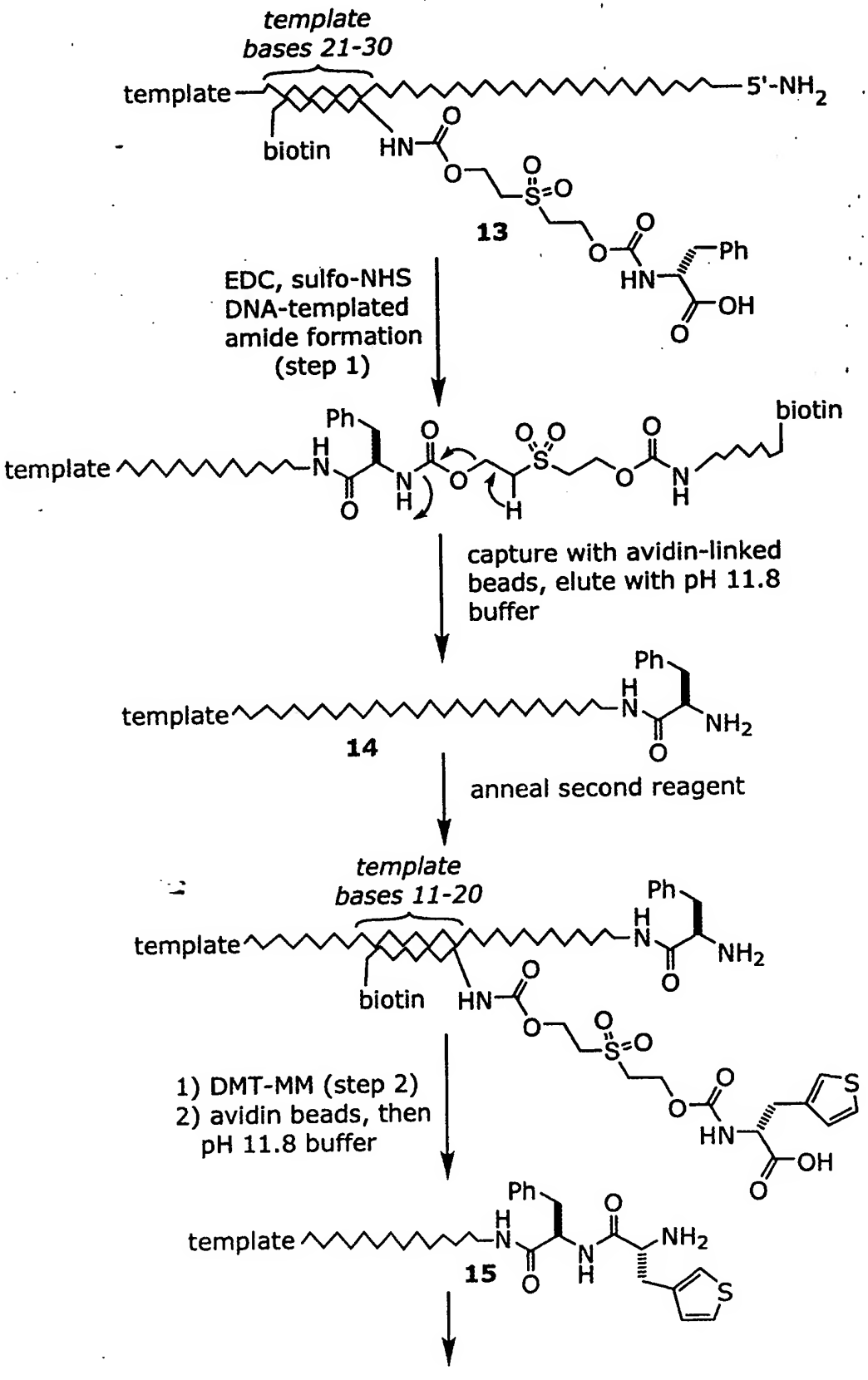

1 only
1 + matched template
1 + mismatched template
4 + matched template
4 + mismatched template
7 + matched template
product **7** after pH 11.8 cleavage
10 + matched template
10 + mismatched template
product **12** after $NaIO_4$ cleavage.
3
6
5
8
9
5
11
12
5
stained with ethidium bromide
unstained (dansylated species only are visible)

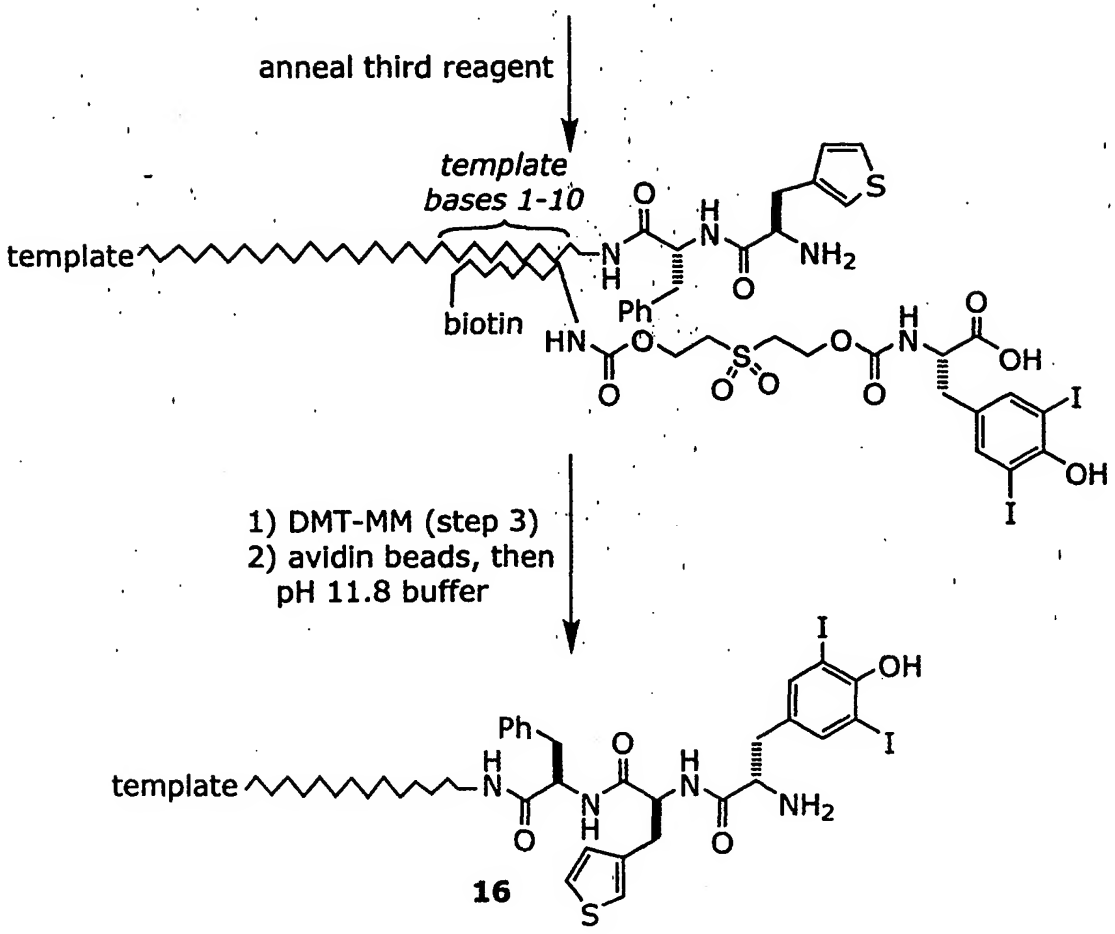
30 A

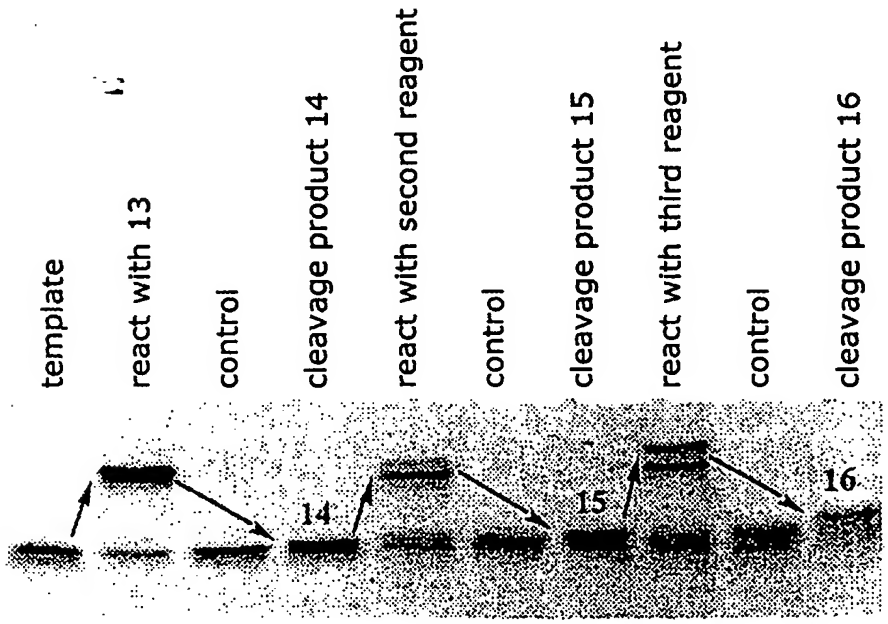

biotin
reagent
R
template
molecule
product and
unreacted template
enter the next step
molecule
R
biotin
product
+
template
avidin
biotin
product
+
biotin
+
template
+
reagent
R
+
avidin
biotin
DNA-templated
synthesis using an
autocleaving linker
wash
with avidin-
linked beads

30 B

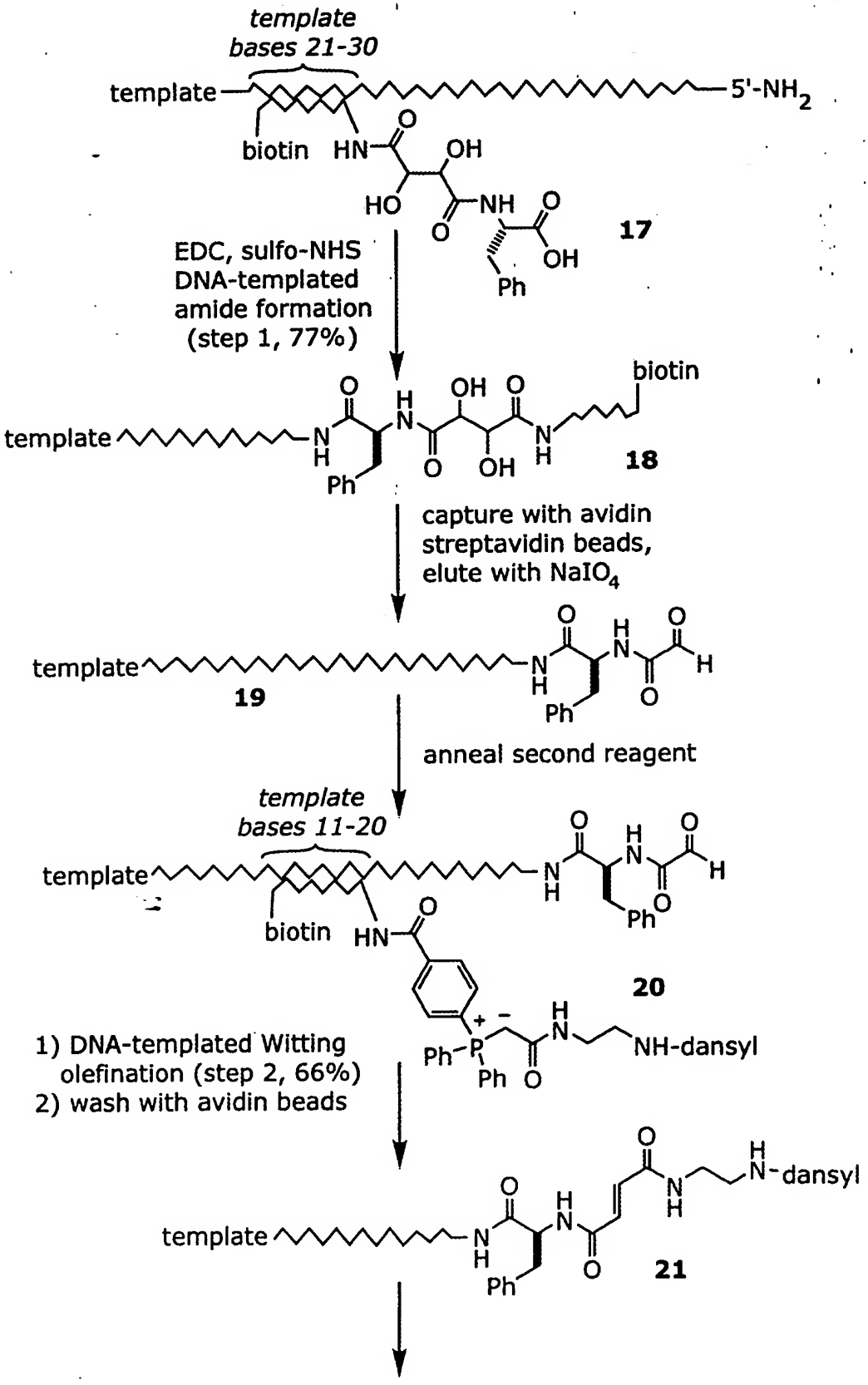

biotin
reagent
R
template
molecule
elute product by
linker cleavage
molecule
R
biotin
product
avidin
biotin
+
R
avidin
biotin
product
biotin
+
template
+
reagent
template
DNA-templated
synthesis using
a traceless linker
or useful scar linker
capture
with avidin-
linked beads

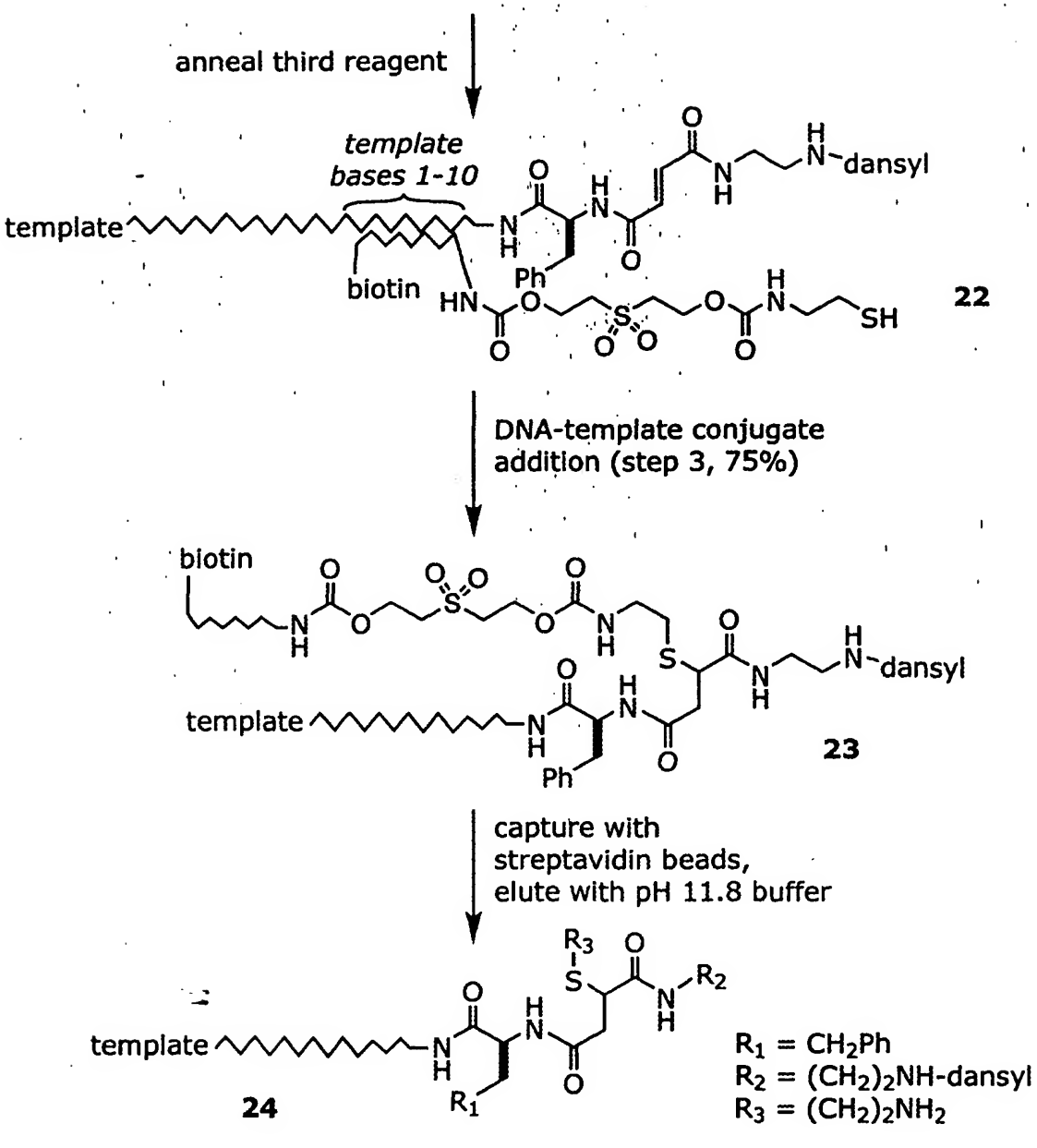

template
bases 21-30
template
5'-NH2
biotin
HN
13
EDC, sulfo-NHS
DNA-templated
amide formation
(step 1)
Ph
OH
biotin
template
capture with avidin-linked
beads, elute with pH 11.8
buffer
template
14
NH2
anneal second reagent
template
bases 11-20
template
biotin
HN
1) DMT-MM (step 2)
2) avidin beads, then
pH 11.8 buffer
template
15
NH2
S

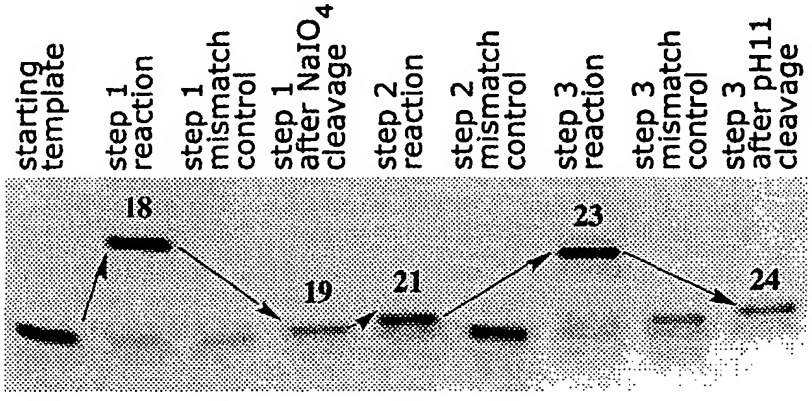

anneal third reagent
template
bases 1-10
template
biotin
1) DMT-MM (step 3)
2) avidin beads, then
pH 11.8 buffer
template
16


template
react with 13
control
cleavage product 14
react with second reagent
control
cleavage product 15
react with third reagent
control
cleavage product 16
14
15
16

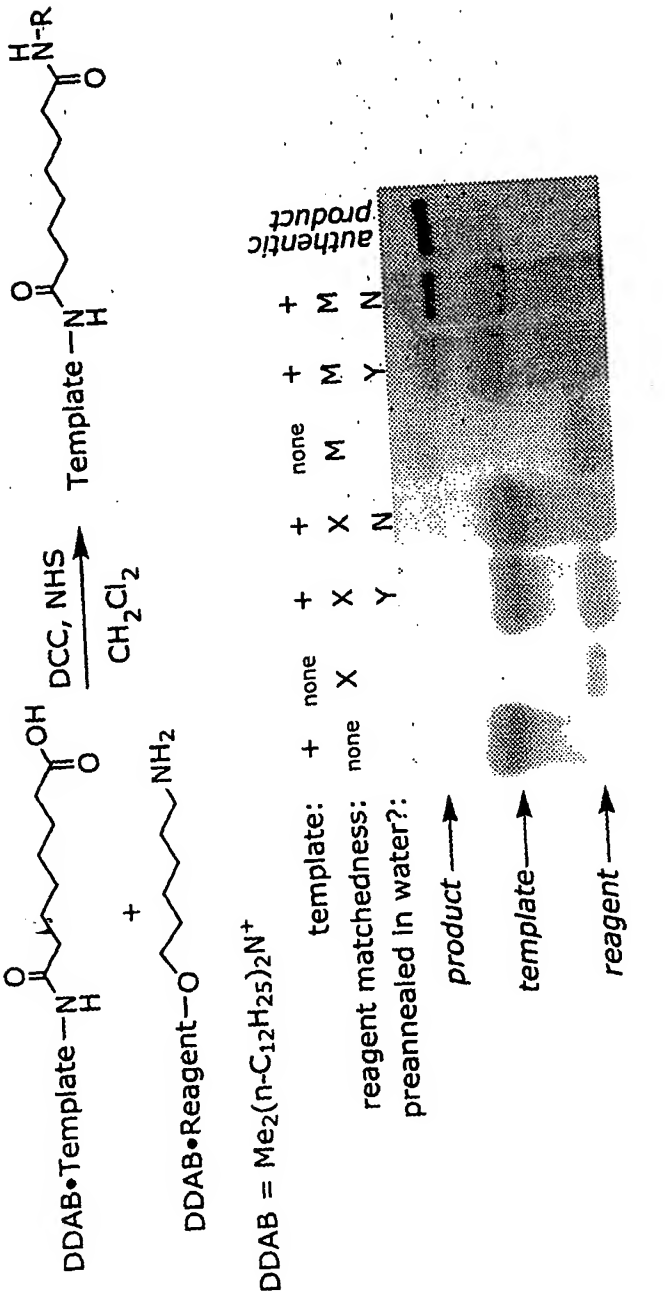
31B


template
bases 21-30
5'-NH2
biotin
HN
OH
HO
NH
OH
Ph
17
EDC, sulfo-NHS
DNA-templated
amide formation
(step 1, 77%)
biotin
template
18
capture with avidin
streptavidin beads,
elute with NaIO4
template
19
anneal second reagent
template
bases 11-20
template
biotin
HN
20
1) DNA-templated Witting
olefination (step 2, 66%)
2) wash with avidin beads
NH-dansyl
dansyl
template
21

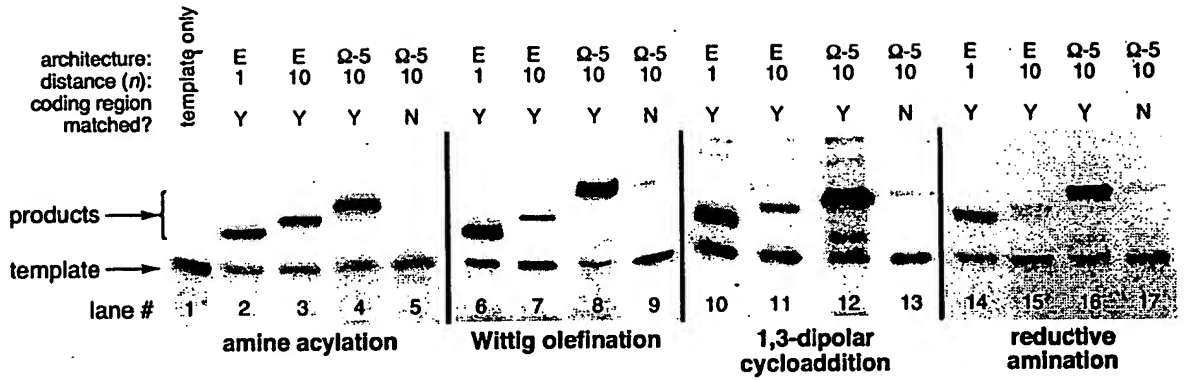

anneal third reagent
template
bases 1-10
template
biotin
dansyl
22
DNA-template conjugate
addition (step 3, 75%)
biotin
dansyl
template
23
capture with
streptavidin beads,
elute with pH 11.8 buffer
template
24
R1 = CH2Ph
R2 = (CH2)2NH-dansyl
R3 = (CH2)2NH2


starting template
step 1 reaction
step 1 mismatch control
step 1 after NaIO4 cleavage
step 2 reaction
step 2 mismatch control
step 3 reaction
step 3 mismatch control
step 3 after pH11 cleavage
18
19
21
23
24

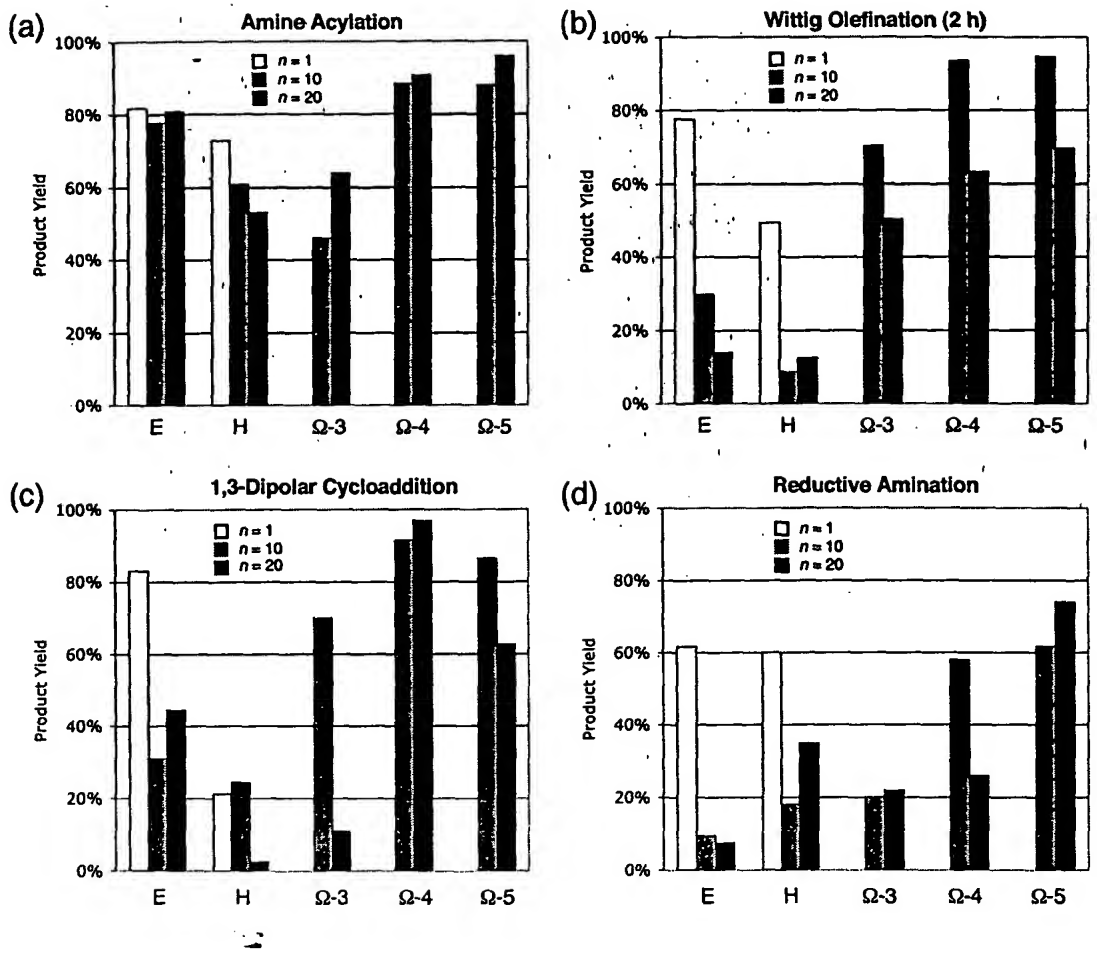
32B


DDAB•Template
DDAB•Reagent
DDAB = $Me_2(n\text{-}C_{12}H_{25})_2N^+$
DCC, NHS
CH_2Cl_2
Template
template:
reagent matchedness:
preannealed in water?:
product
template
reagent
authentic product

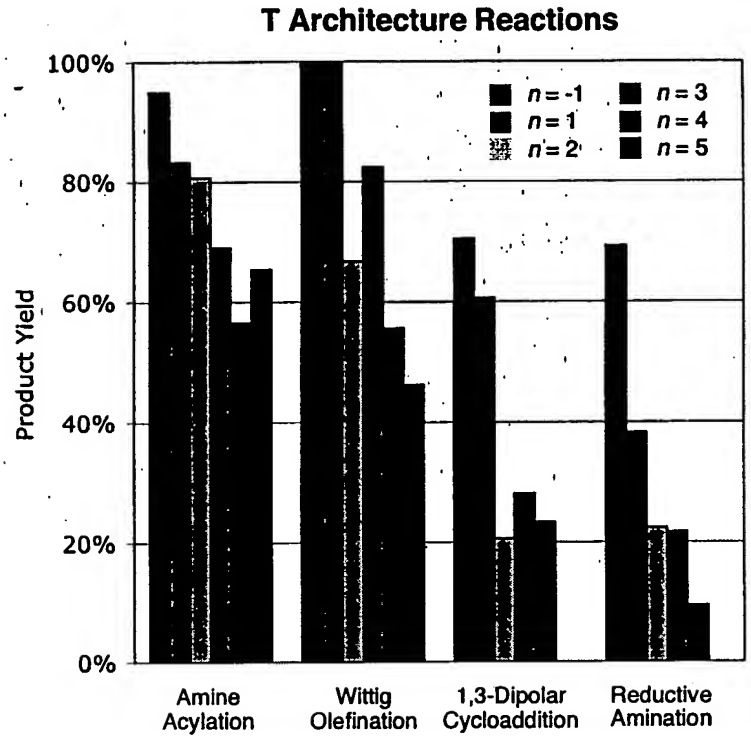
33

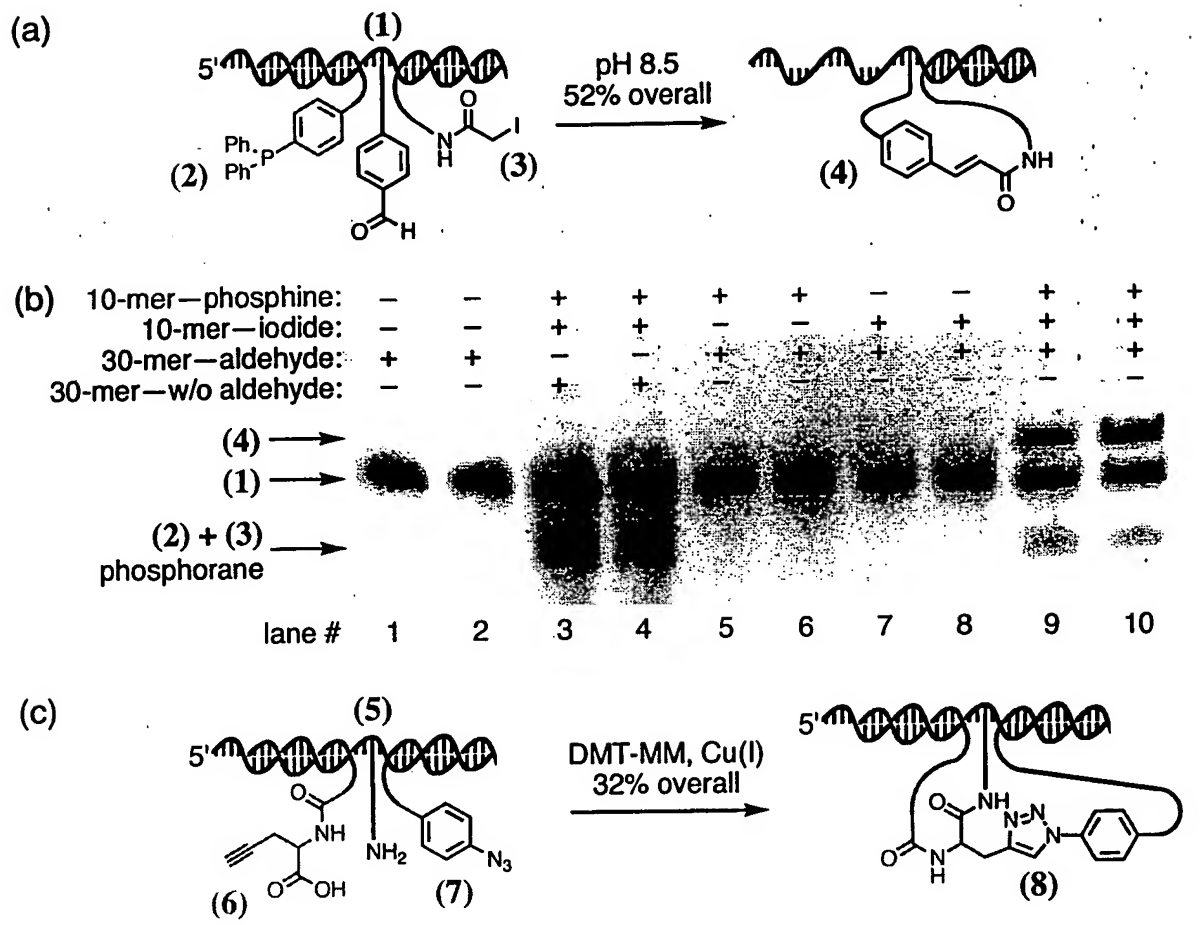

architecture:
distance (n):
coding region matched?
template only
E 1 Y
E 10 Y
Ω-5 10 Y
Ω-5 10 N
E 1 Y
E 10 Y
Ω-5 10 Y
Ω-5 10 N
E 1 Y
E 10 Y
Ω-5 10 Y
Ω-5 10 N
E 1 Y
E 10 Y
Ω-5 10 Y
Ω-5 10 N
products
template
lane # 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
amine acylation
Wittig olefination
1,3-dipolar cycloaddition
reductive amination

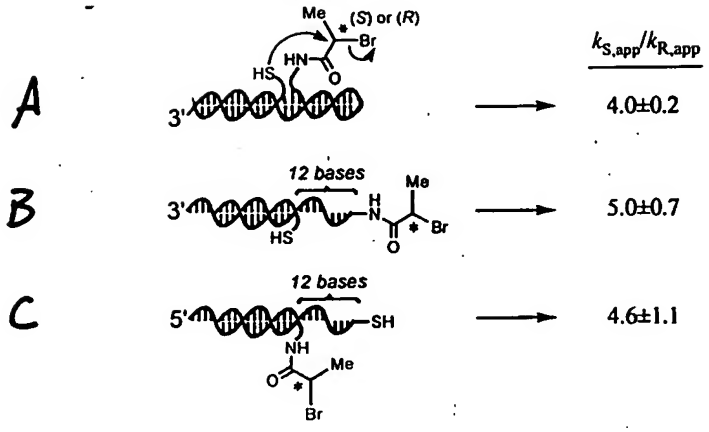
34

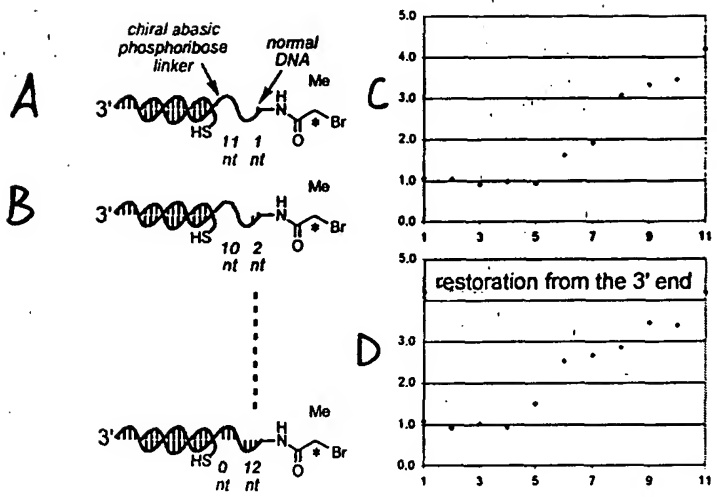

(a)
Amine Acylation
(b)
Wittig Olefination (2 h)
(c)
1,3-Dipolar Cycloaddition
(d)
Reductive Amination
n = 1
n = 10
n = 20
Product Yield
100%
80%
60%
40%
20%
0%
E
H
Ω-3
Ω-4
Ω-5

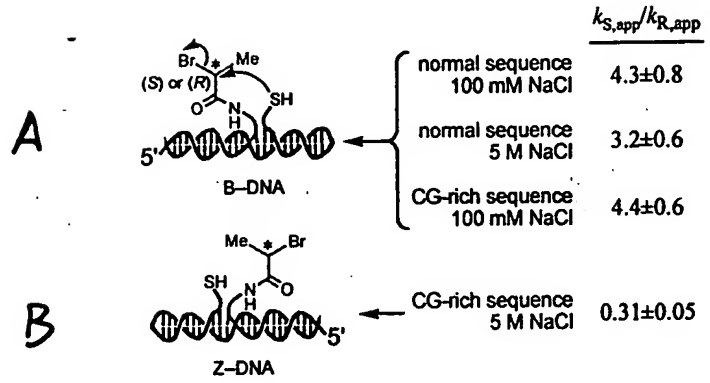
Architecture	Buffer	T_m (°C)
E (*n*=10)	PBS	45
Ω (*n*=10)	PBS	46
E (*n*=10)	HSP	55
Ω (*n*=10)	HSP	54
E (*n*=20)	PBS	40
Ω (*n*=20)	PBS	39


T Architecture Reactions
Product Yield
100%
80%
60%
40%
20%
0%
n = -1
n = 1
n = 2
n = 3
n = 4
n = 5
Amine Acylation
Wittig Olefination
1,3-Dipolar Cycloaddition
Reductive Amination

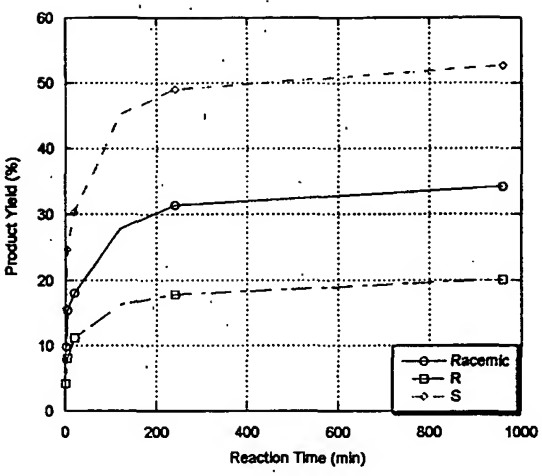

(a)
(1)
5'
pH 8.5
52% overall
(2)
(3)
(4)
(b)
10-mer—phosphine:
10-mer—iodide:
30-mer—aldehyde:
30-mer—w/o aldehyde:
(4)
(1)
(2) + (3)
phosphorane
lane # 1 2 3 4 5 6 7 8 9 10
(c)
(5)
5'
DMT-MM, Cu(I)
32% overall
(6)
(7)
(8)


A
B
C
Me
(S) or (R)
Br
HS
HN
O
3'
12 bases
H
N
Me
Br
HS
O
5'
SH
NH
Me
O
Br
$k_{S,app}/k_{R,app}$
4.0±0.2
5.0±0.7
4.6±1.1

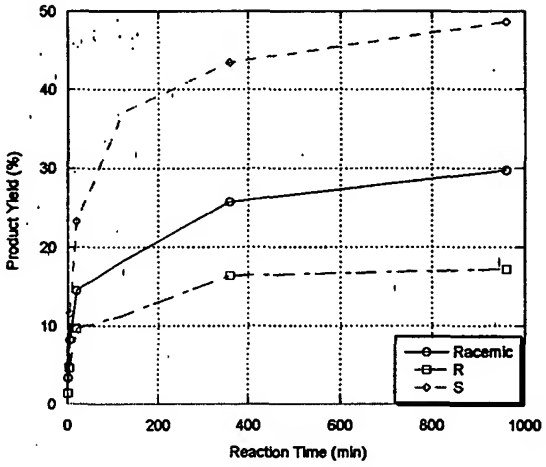

A
B
chiral abasic phosphoribose linker
normal DNA
3'
HS
Me
Br
11 nt
1 nt
10 nt
2 nt
0 nt
12 nt
C
D
restoration from the 3' end
5.0
4.0
3.0
2.0
1.0
0.0
1
3
5
7
9
11


$k_{S,app}/k_{R,app}$
A
Br
Me
(S) or (R)
SH
O
N
H
5'
B–DNA
normal sequence
100 mM NaCl
4.3±0.8
normal sequence
5 M NaCl
3.2±0.6
CG-rich sequence
100 mM NaCl
4.4±0.6
B
Me
Br
SH
N
H
O
5'
Z–DNA
CG-rich sequence
5 M NaCl
0.31±0.05

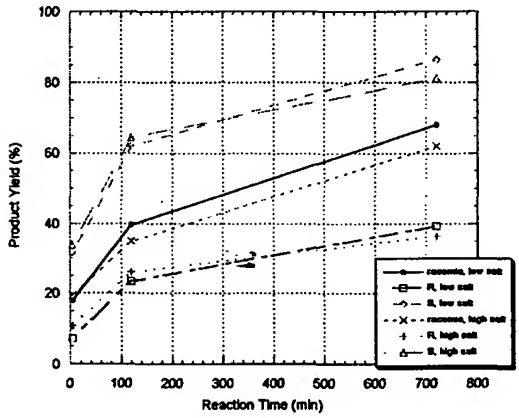
A


Product Yield (%)
Reaction Time (min)
Racemic
R
S

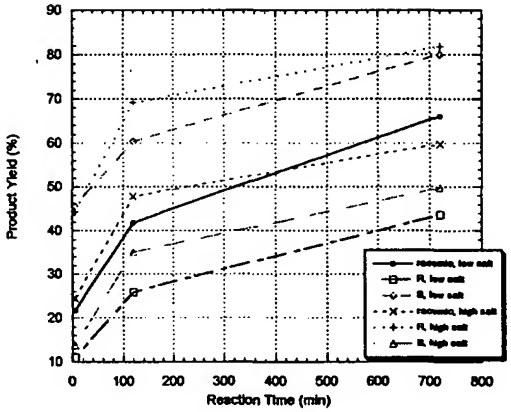
B

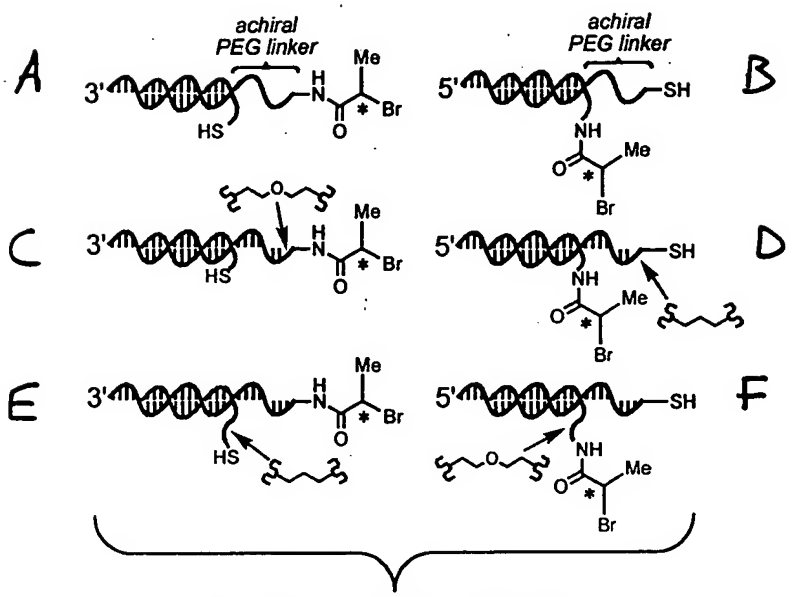

Product Yield (%)
Reaction Time (min)
Racemic
R
S

C


Product Yield (%)
Reaction Time (min)

D

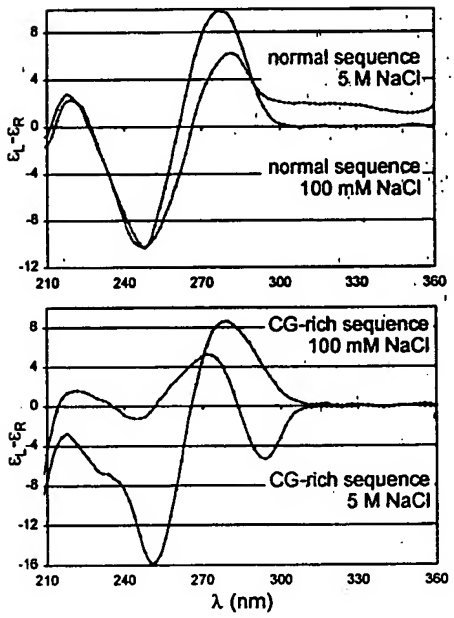

Product Yield (%)
Reaction Time (min)



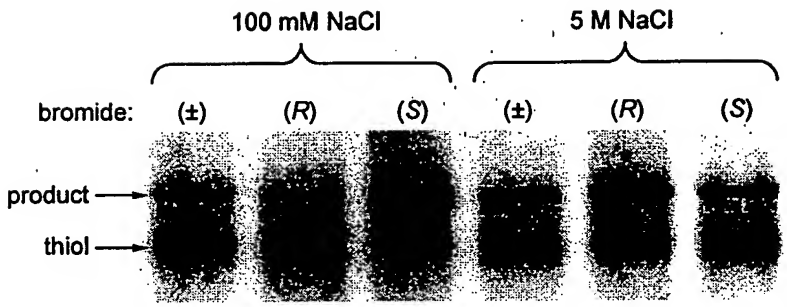
$k_{S,app}/k_{R,app}$ = 0.95 to 1.09±0.09

A

B


normal sequence
5 M NaCl
normal sequence
100 mM NaCl
CG-rich sequence
100 mM NaCl
CG-rich sequence
5 M NaCl
ε_L-ε_R
λ (nm)
8
4
0
-4
-8
-12
-16
210
240
270
300
330
360

44


100 mM NaCl
5 M NaCl
bromide:
(±)
(R)
(S)
(±)
(R)
(S)
product
thiol

SEQ ID NO:

templates

14 3'-TTAAGCATGGT-R (11-mer) 1a-1c

15 3'-TCTGATAGAGAGCAATT-R (17-mer) 2a-2c

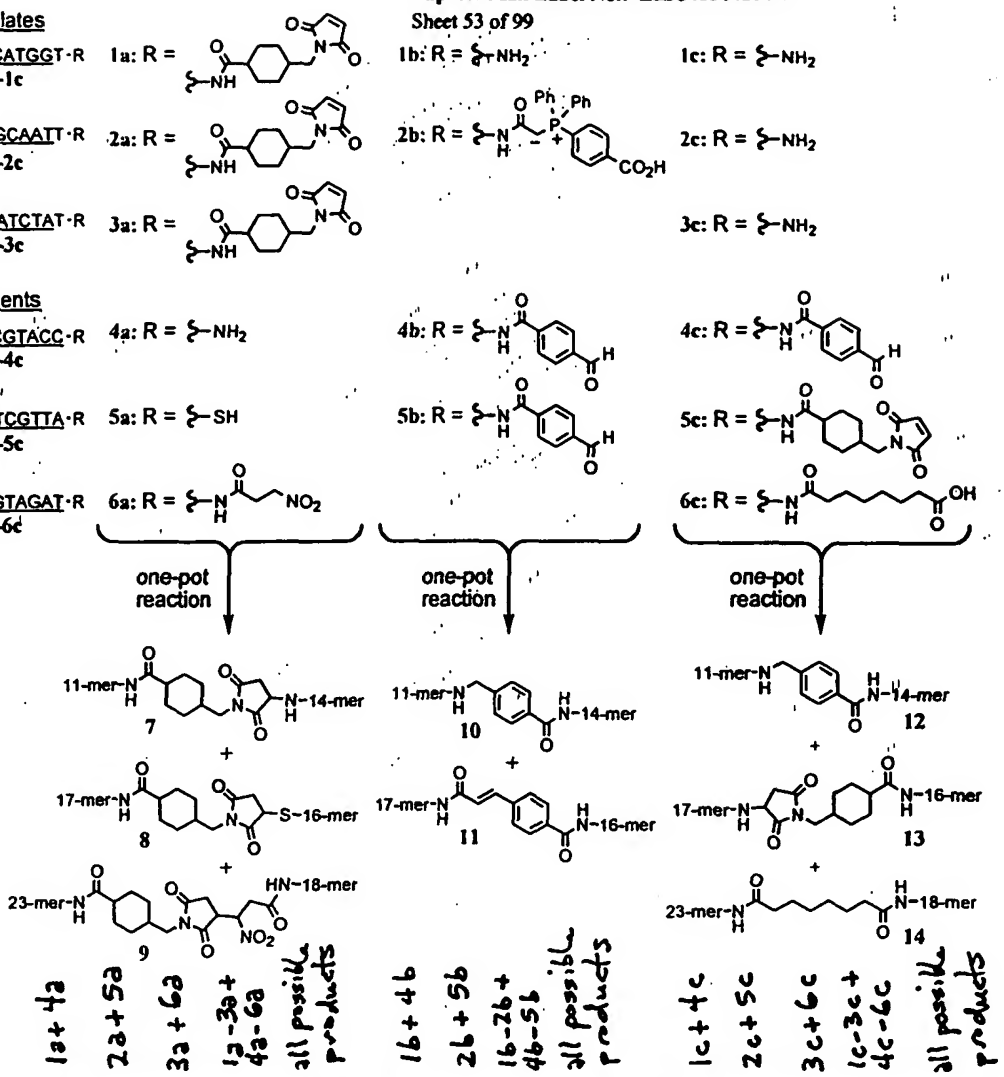
16 3'-CAGTAATCTGATGAGACATCTAT-R (23-mer) 3a-3c

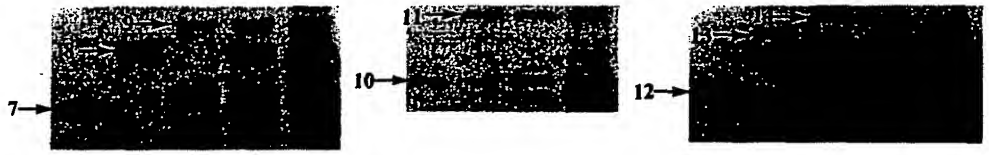
reagents

17 5'-CAGCAATTCGTACC-R (14-mer) 4a-4c

18 5'-CTCAGCTCTCTCGTTA-R (16-mer) 5a-5c

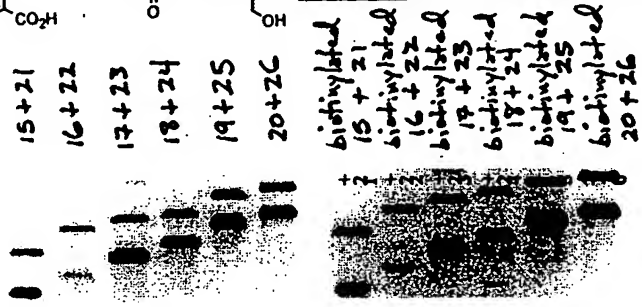
19 5'-GGCTCAGCCTCTGTAGAT-R (18-mer) 6a-6c

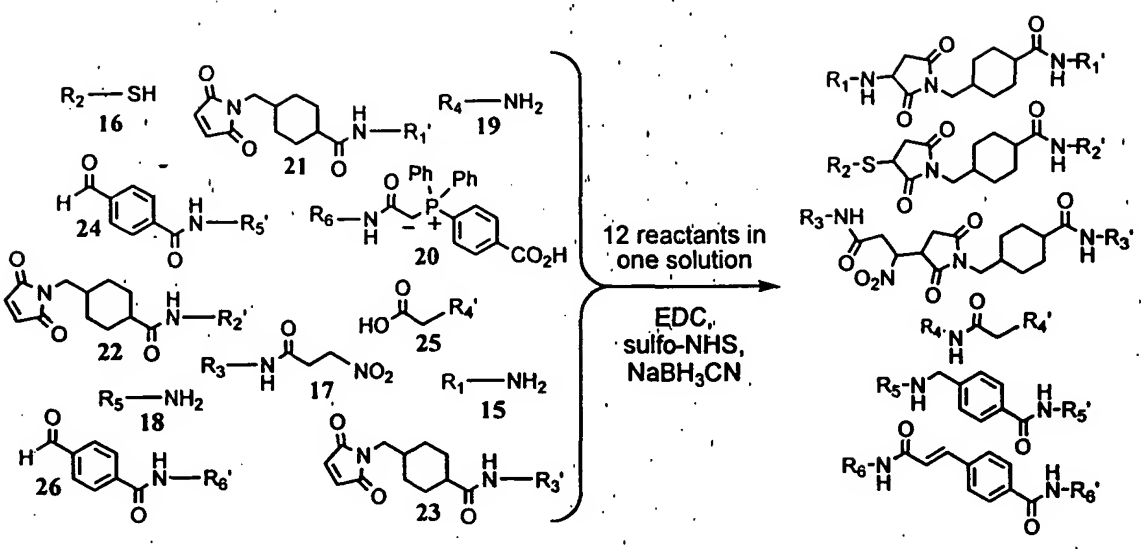




Eq ID NO:		templates	reagents		SEQ ID NO:
20	15	3'-CGACTAGATAT-O-(O)-NH$_2$	O-TATCTAGTCGCGACT-5'	21	25
14	16	3'-TTAAGCATGGT-O-(O)-N(H)-SH	O-CCATGCTTAACGACTCGGGA-5'	22	26
21	17	3'-AGAGAGCAATT-O-(O)-N(H)-NO$_2$	O-ATTGCTCTCTCGACTCGGGAATGCA-5'	23	27
22	18	3'-GAGACATCTAT-NH$_2$	O-TAGATGTCTCCGACTCGGGAATGCCGACCTT-5'	24	28
23	19	3'-AATGTAGTCCT-O-(O)-NH$_2$	O-GGACTACATTCGACTCGGGAATCCAGCCTTTACGG-5'	25	29
24	20	3'-TCGTCTAGAAT-O-(O)-N(H)-P(Ph)(Ph)-CO$_2$H	O-TTCTAGACGACGACTCGGGAATGCAGCCTTTACGGTATCT-5'	26	30

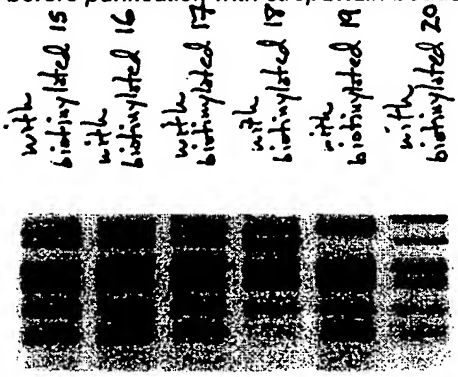
pairwise reactions (one template, one reagent):



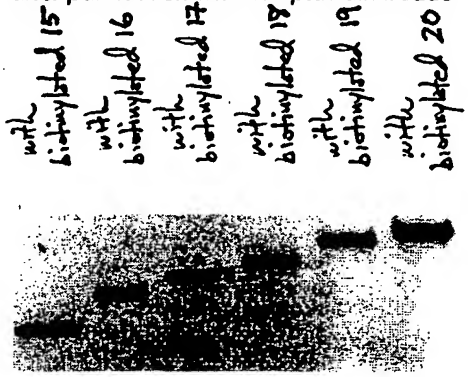


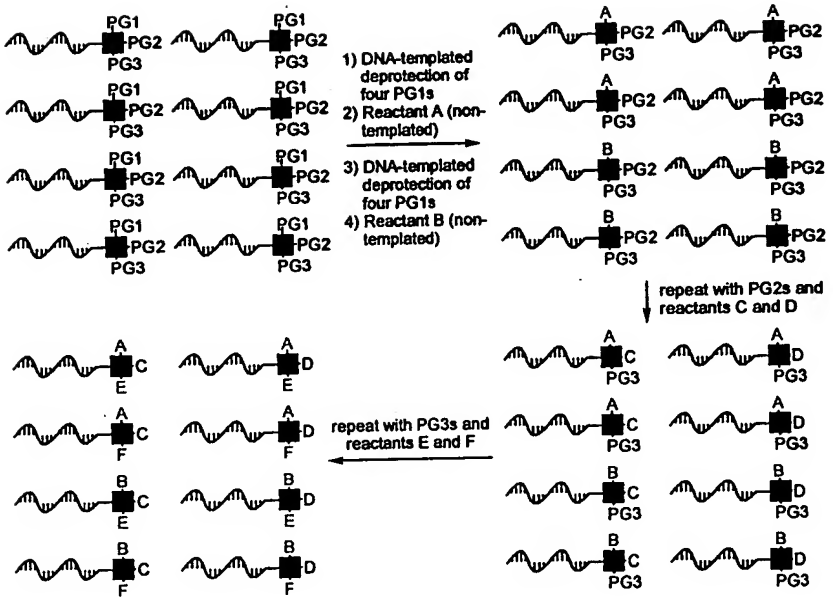
one-pot reactions containing one biotinylated template (**15**, **16**, **17**, **18**, **19**, or **20**) + five non-biotinylated templates (out of **15-20**) + six reagents (**21-26**)

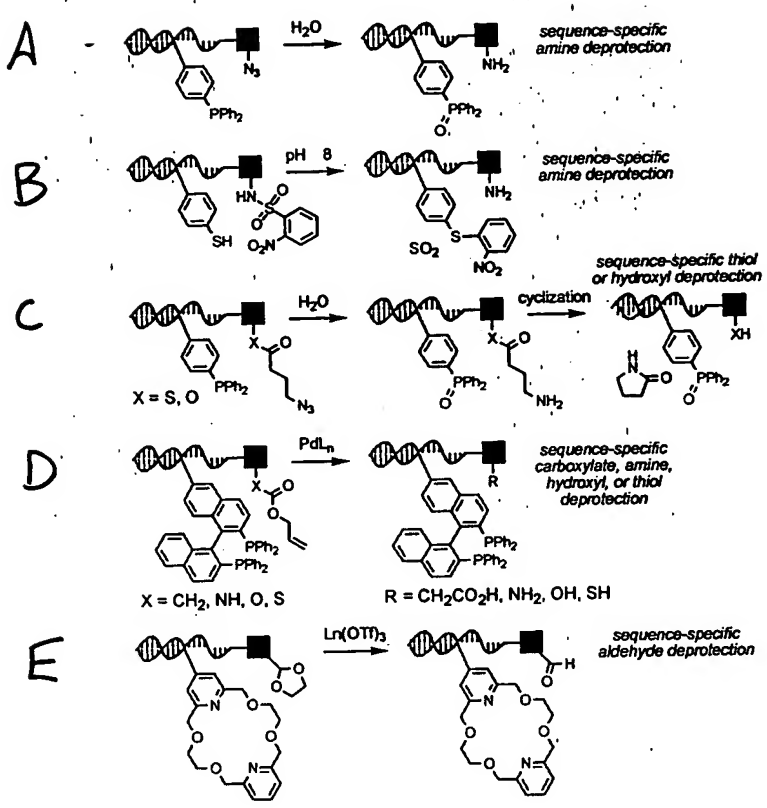
before purification with strepavidin beads



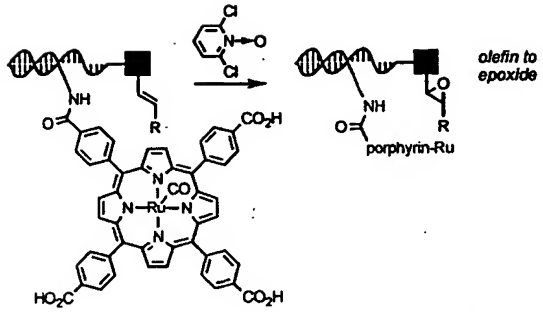
after purification with streptavidin beads



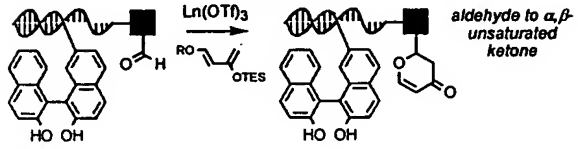

PG1
PG2
PG3
1) DNA-templated deprotection of four PG1s
2) Reactant A (non-templated)
3) DNA-templated deprotection of four PG1s
4) Reactant B (non-templated)
A
B
repeat with PG2s and reactants C and D
C
D
repeat with PG3s and reactants E and F
E
F

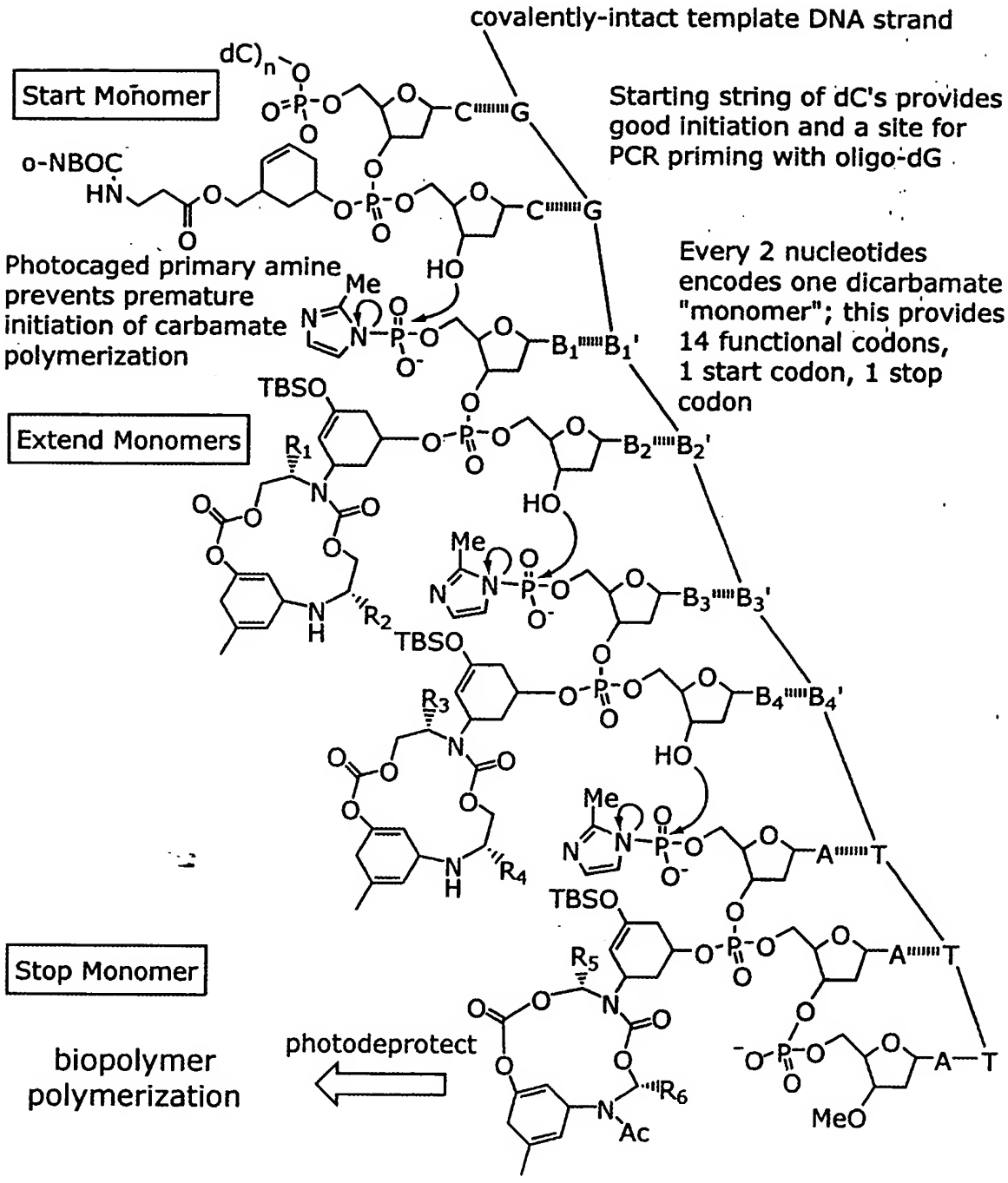

A
H_2O
sequence-specific amine deprotection
B
pH 8
sequence-specific amine deprotection
C
H_2O
cyclization
sequence-specific thiol or hydroxyl deprotection
X = S, O
D
PdL_n
sequence-specific carboxylate, amine, hydroxyl, or thiol deprotection
X = CH_2, NH, O, S
R = CH_2CO_2H, NH_2, OH, SH
E
$Ln(OTf)_3$
sequence-specific aldehyde deprotection

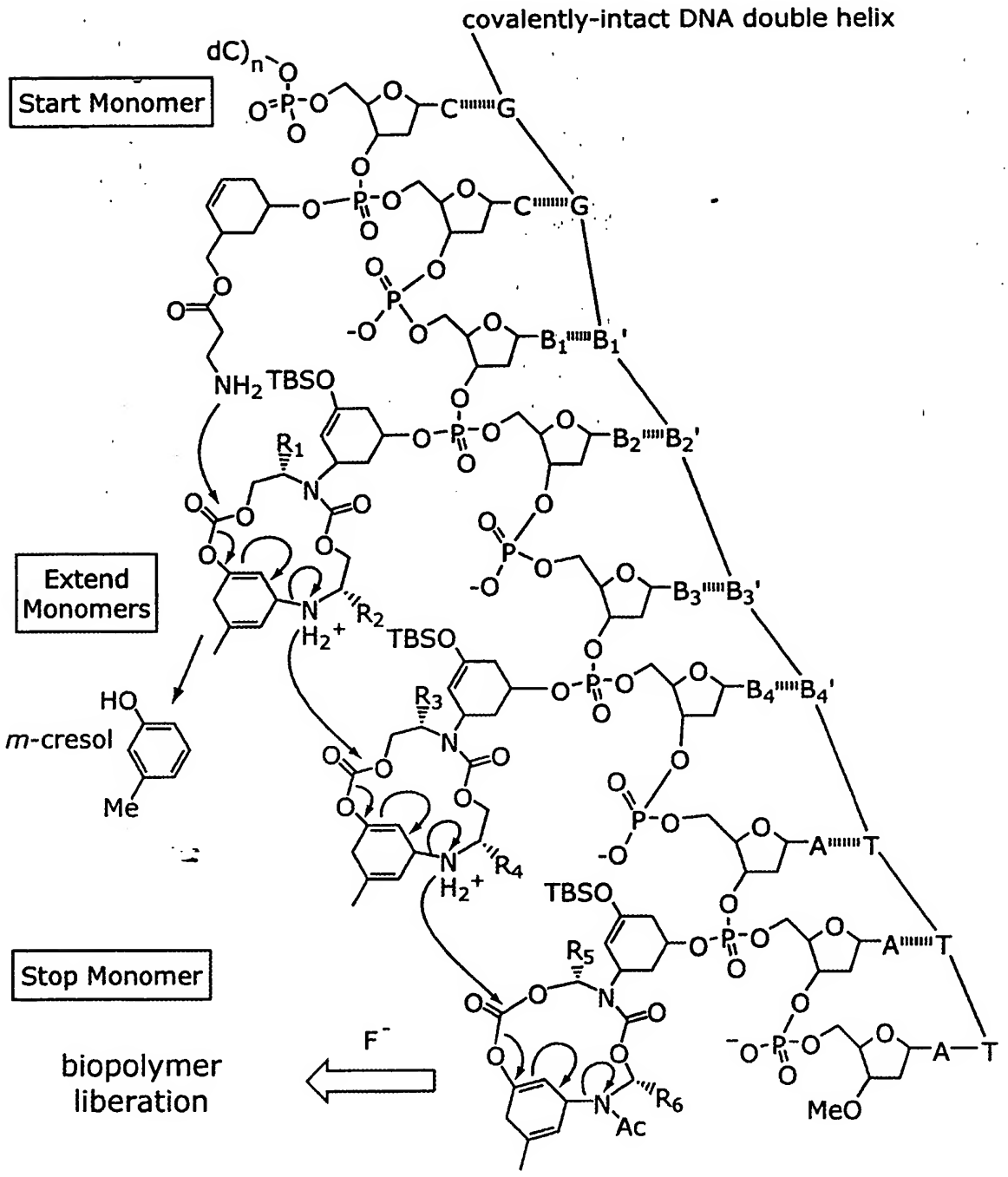
A

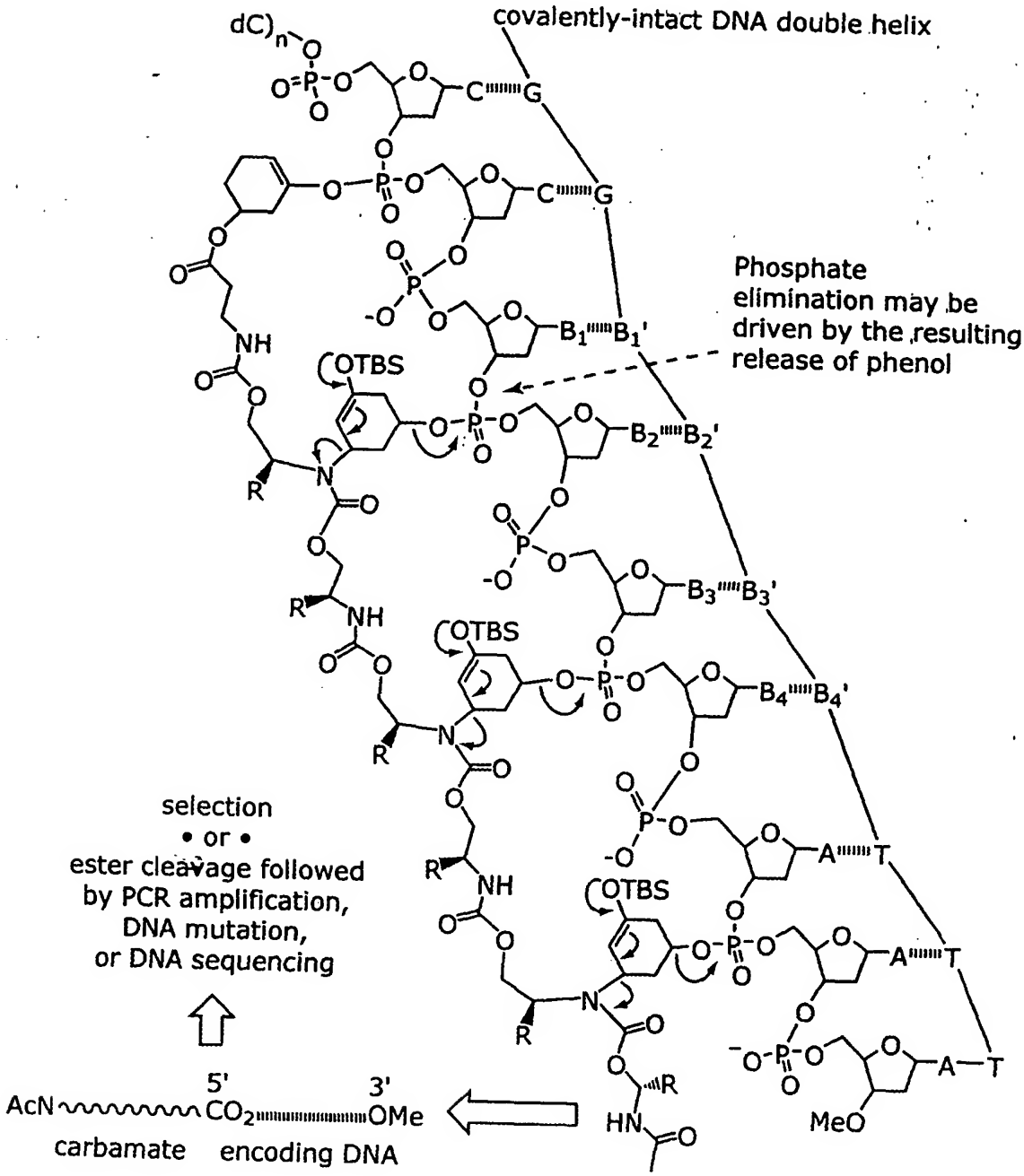

Cl
N→O
Cl
NH
O
R
CO2H
CO
N
Ru
HO2C
CO2H
olefin to epoxide
O
NH
O
R
porphyrin-Ru

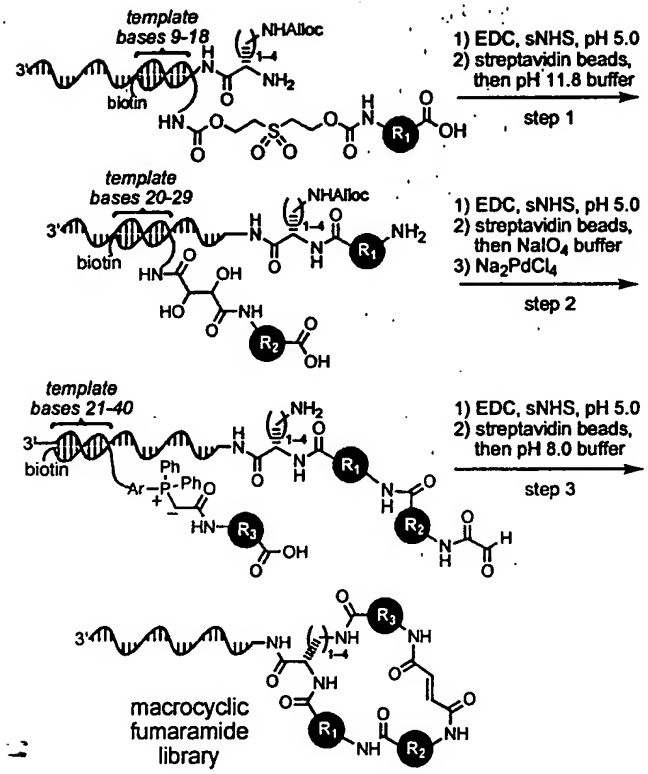
B

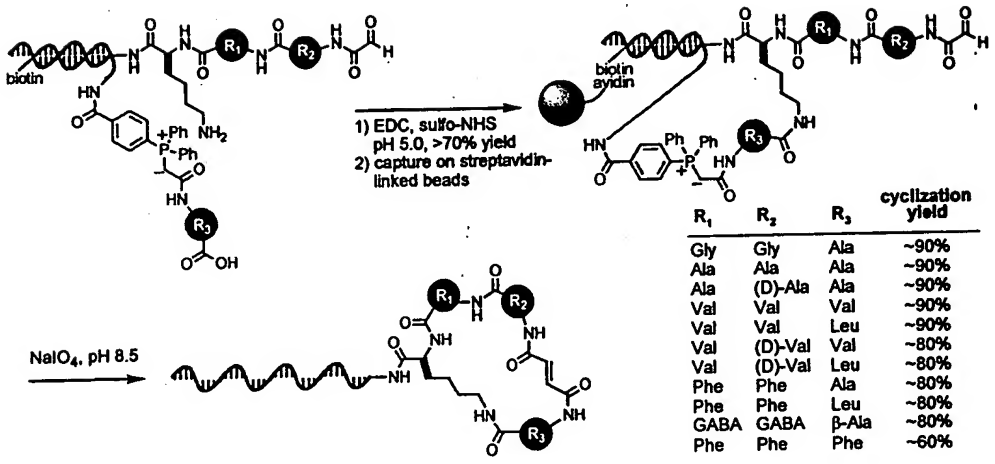

Ln(OTf)3
O
H
RO
OTES
HO OH
aldehyde to α,β-unsaturated ketone
O
O
HO OH


covalently-intact template DNA strand
Start Monomer
Starting string of dC's provides good initiation and a site for PCR priming with oligo-dG
Photocaged primary amine prevents premature initiation of carbamate polymerization
Every 2 nucleotides encodes one dicarbamate "monomer"; this provides 14 functional codons, 1 start codon, 1 stop codon
Extend Monomers
Stop Monomer
biopolymer polymerization
photodeprotect

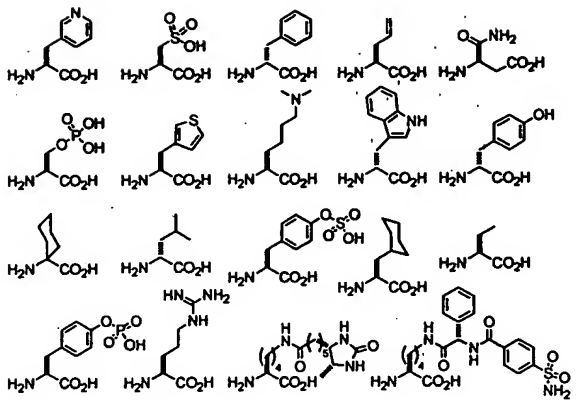

covalently-intact DNA double helix
Start Monomer
Extend Monomers
Stop Monomer
biopolymer liberation
F⁻
m-cresol
HO
Me
MeO
TBSO
NH2
Ac

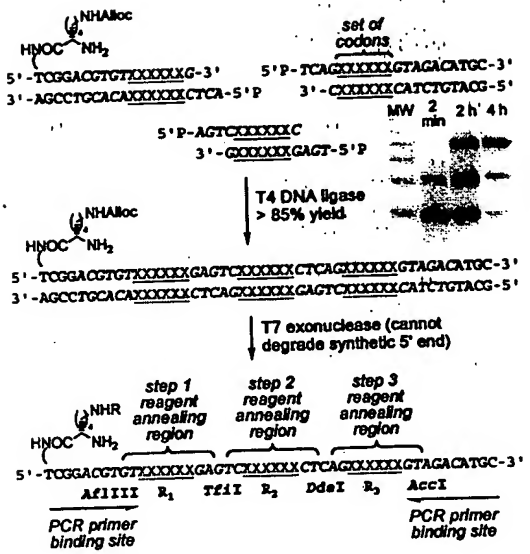

covalently-intact DNA double helix
Phosphate elimination may be driven by the resulting release of phenol
selection • or • ester cleavage followed by PCR amplification, DNA mutation, or DNA sequencing
5'
3'
AcN
CO2
OMe
carbamate
encoding DNA
OTBS
MeO


template
bases 9-18
NHAlloc
biotin
1) EDC, sNHS, pH 5.0
2) streptavidin beads,
then pH 11.8 buffer
step 1
template
bases 20-29
NHAlloc
biotin
1) EDC, sNHS, pH 5.0
2) streptavidin beads,
then NaIO4 buffer
3) Na2PdCl4
step 2
template
bases 21-40
biotin
1) EDC, sNHS, pH 5.0
2) streptavidin beads,
then pH 8.0 buffer
step 3
macrocyclic
fumaramide
library

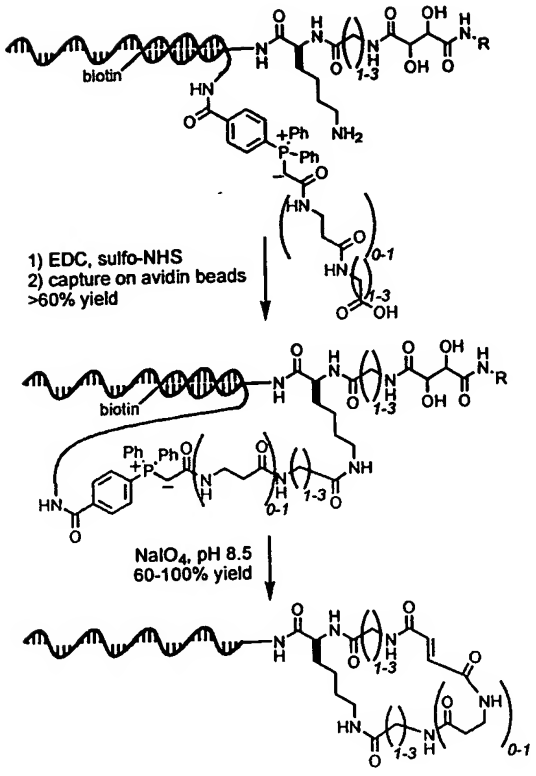


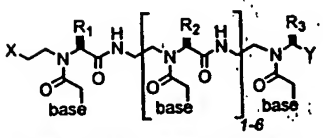
R_1	R_2	R_3	cyclization yield
Gly	Gly	Ala	~90%
Ala	Ala	Ala	~90%
Ala	(D)-Ala	Ala	~90%
Val	Val	Val	~90%
Val	Val	Leu	~90%
Val	(D)-Val	Val	~80%
Val	(D)-Val	Leu	~80%
Phe	Phe	Ala	~80%
Phe	Phe	Leu	~80%
GABA	GABA	β-Ala	~80%
Phe	Phe	Phe	~60%




NHAlloc
HNOC NH2
set of codons
5'-TCGGACGTGTXXXXXG-3'
5'P-TCAGXXXXXGTAGACATGC-3'
3'-AGCCTGCACAXXXXXCTCA-5'P
3'-GXXXXXCATCTGTACG-5'
MW 2 min 2h 4h
5'P-AGTCXXXXXC
3'-GXXXXXGAGT-5'P
T4 DNA ligase
> 85% yield
NHAlloc
HNOC NH2
5'-TCGGACGTGTXXXXXGAGTCXXXXXCTCAGXXXXXGTAGACATGC-3'
3'-AGCCTGCACAXXXXXCTCAGXXXXXGAGTCXXXXXCATCTGTACG-5'
T7 exonuclease (cannot degrade synthetic 5' end)
NHR
HNOC NH2
step 1 reagent annealing region
step 2 reagent annealing region
step 3 reagent annealing region
5'-TCGGACGTGTXXXXXGAGTCXXXXXCTCAGXXXXXGTAGACATGC-3'
AflIII R1 TfiI R2 DdeI R3 AccI
PCR primer binding site
PCR primer binding site

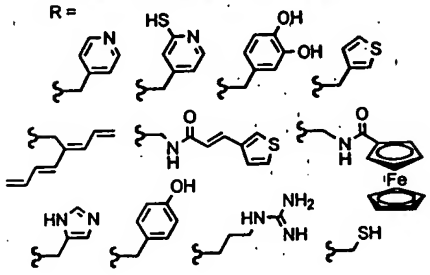
(SEQ ID NO: 31)

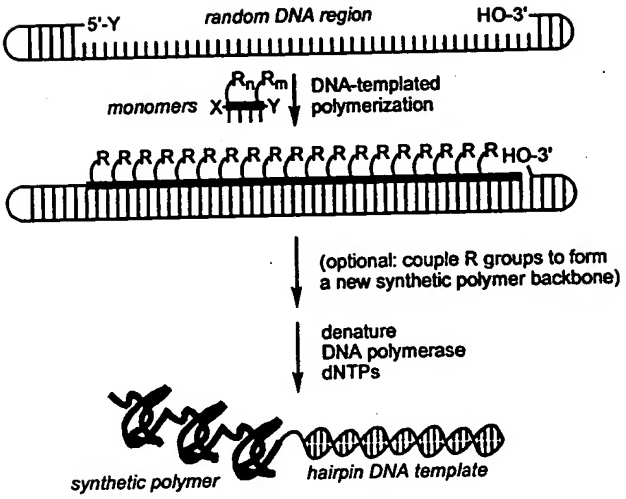

biotin
1) EDC, sulfo-NHS
2) capture on avidin beads
>60% yield
biotin
$NaIO_4$, pH 8.5
60-100% yield

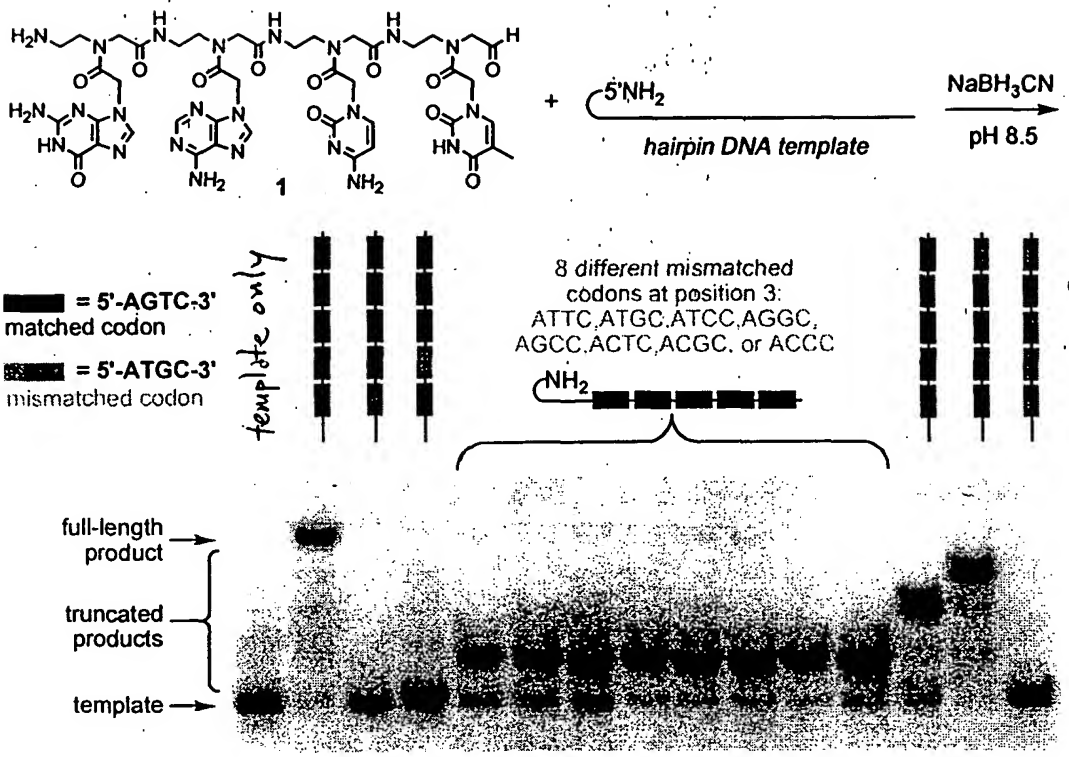


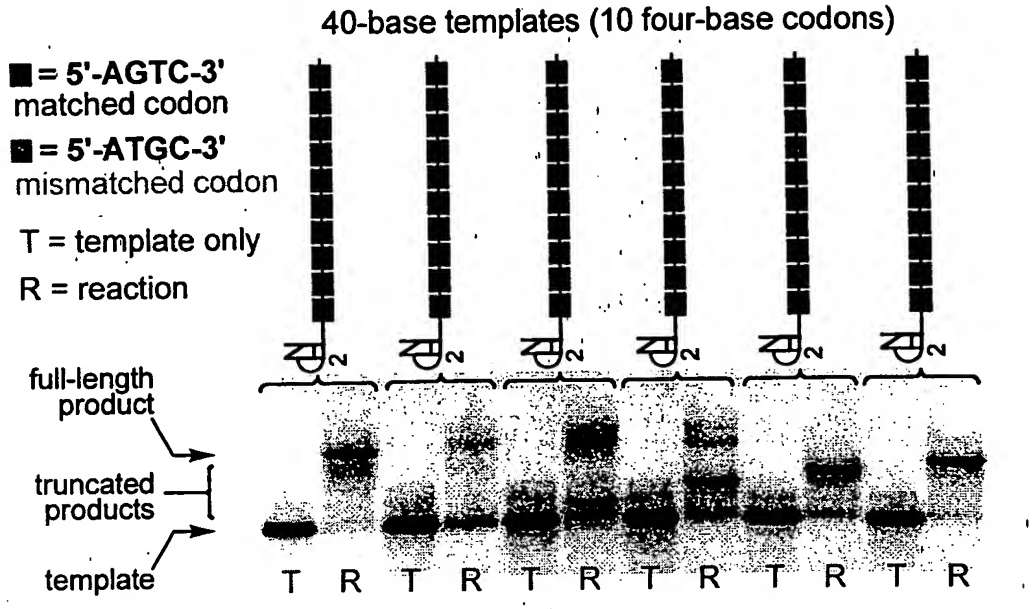
X, Y = groups for coupling (see Fig. 1)

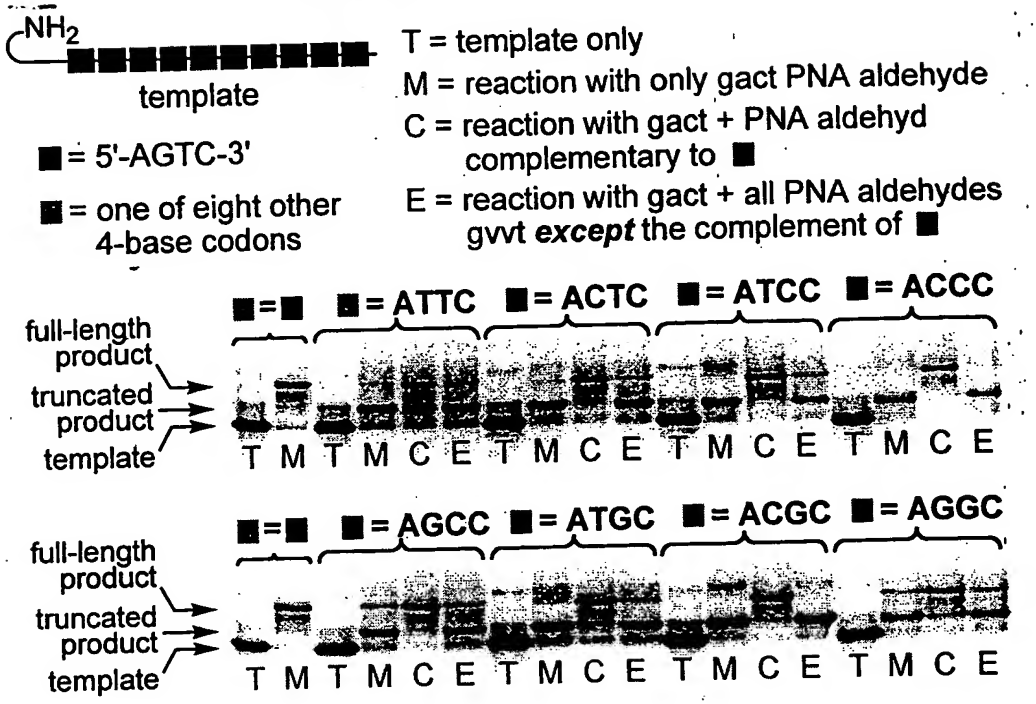
R =




5'-Y
random DNA region
HO-3'
monomers
DNA-templated
polymerization
HO-3'
(optional: couple R groups to form
a new synthetic polymer backbone)
denature
DNA polymerase
dNTPs
synthetic polymer
hairpin DNA template

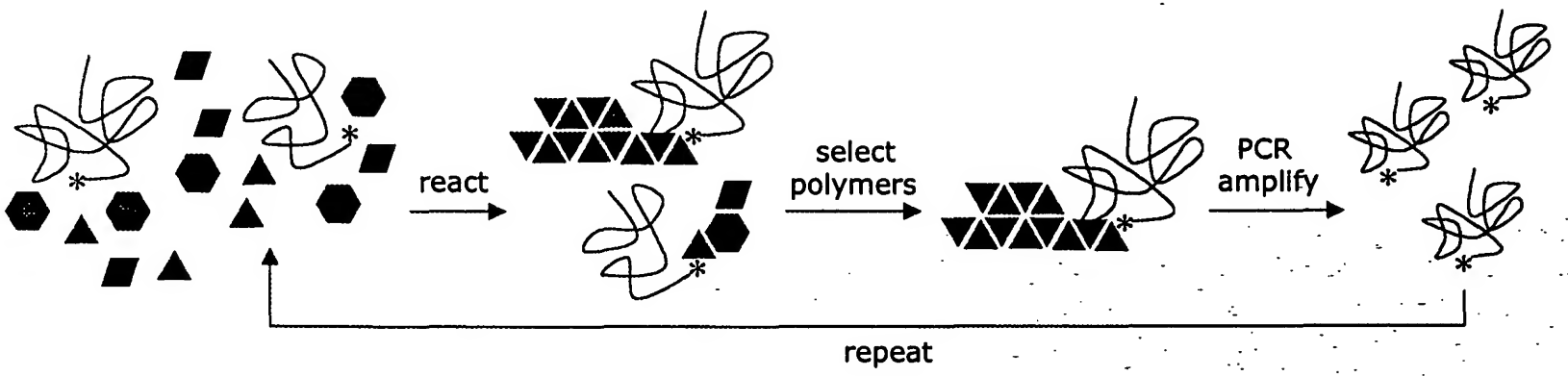

1
5'NH2
hairpin DNA template
NaBH3CN
pH 8.5
= 5'-AGTC-3'
matched codon
= 5'-ATGC-3'
mismatched codon
template only
8 different mismatched
codons at position 3:
ATTC,ATGC,ATCC,AGGC,
AGCC,ACTC,ACGC, or ACCC
NH2
full-length
product
truncated
products
template


40-base templates (10 four-base codons)
■ = 5'-AGTC-3'
matched codon
■ = 5'-ATGC-3'
mismatched codon
T = template only
R = reaction
full-length product
truncated products
template
T R T R T R T R T R T R


NH2
template
■ = 5'-AGTC-3'
■ = one of eight other 4-base codons
T = template only
M = reaction with only gact PNA aldehyde
C = reaction with gact + PNA aldehyd complementary to ■
E = reaction with gact + all PNA aldehydes gvvt *except* the complement of ■
■=■
■ = ATTC
■ = ACTC
■ = ATCC
■ = ACCC
full-length product
truncated product
template
T M T M C E T M C E T M C E T M C E
■=■
■ = AGCC
■ = ATGC
■ = ACGC
■ = AGGC
full-length product
truncated product
template
T M T M C E T M C E T M C E T M C E

Evolving Plastics

- How can amplifiable information be translated into materials with specific properties (e.g., plastics)?
- Nucleic acids can fold into complex structures



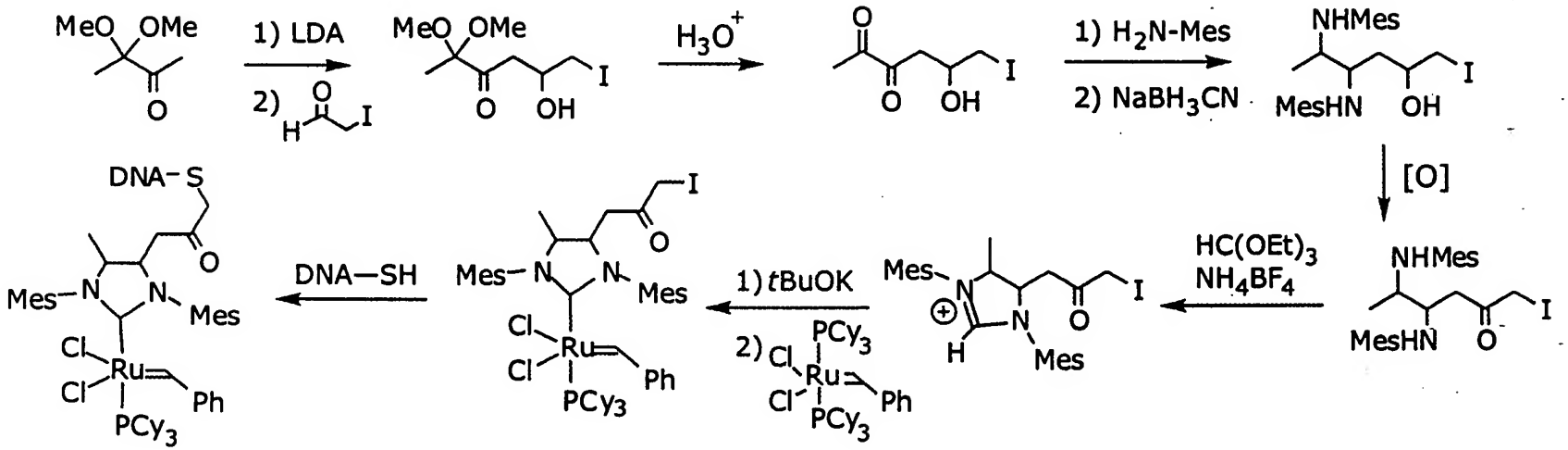
Requirements:

- -Linkage between information and product: need living polymerization
- -Selection for desired materials: gel electrophoresis, sedimentation, mechanical sorting, solvent partitioning
- -Chemical compatibility with DNA: stability in water

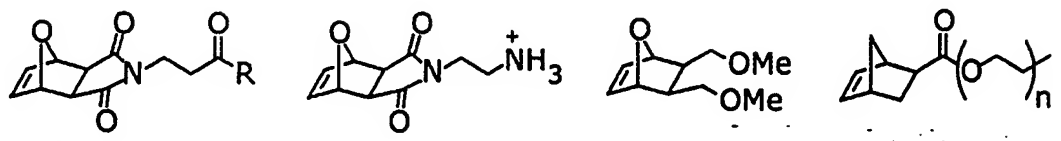
Evolving Plastics

Ring-opening metathesis polymerization (ROMP, R. Grubbs) is mediated by a ruthenium catalyst

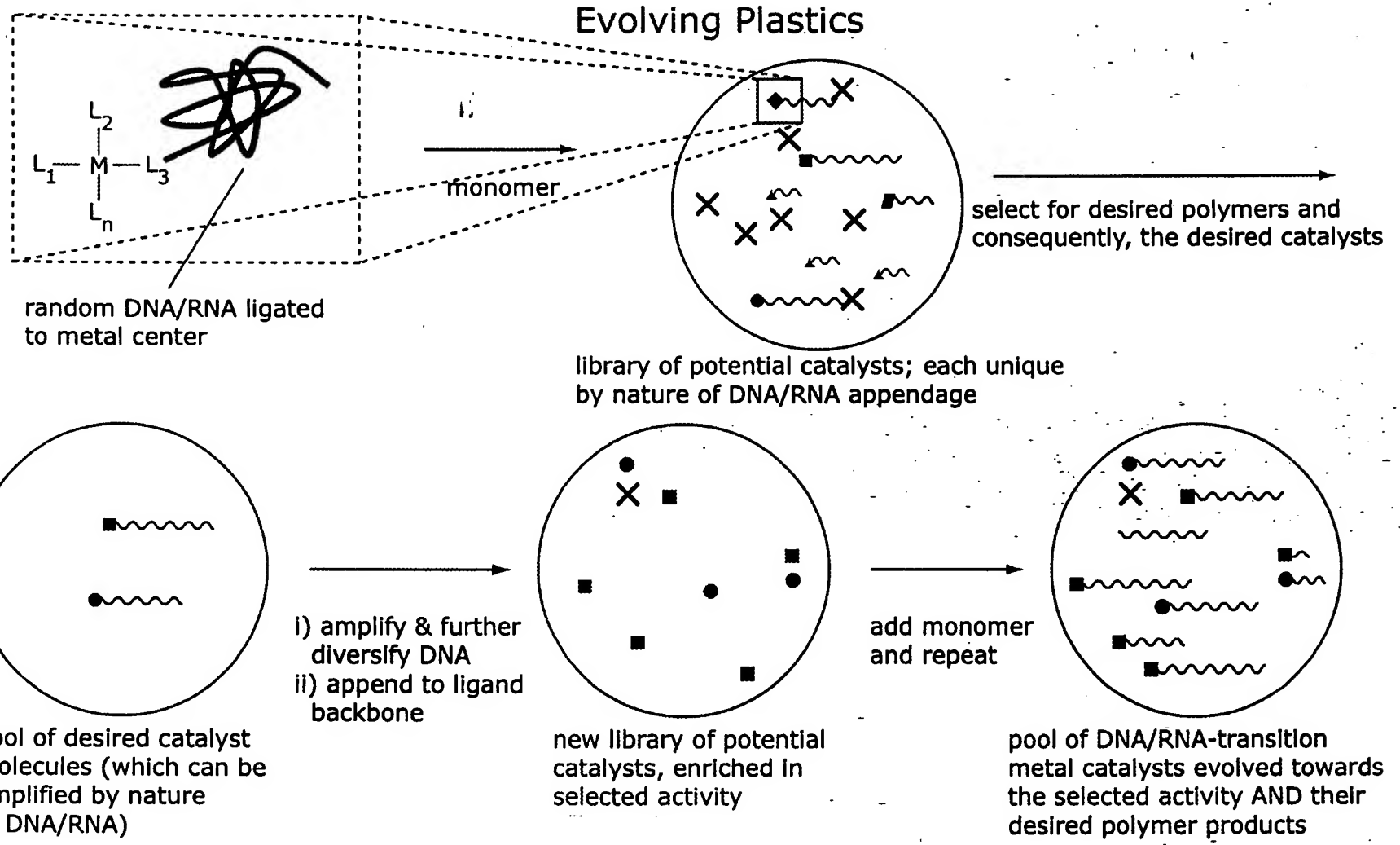
- ROMP is aqueous-compatible and is a living polymerization

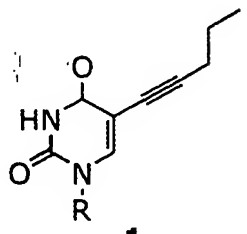


monomers:

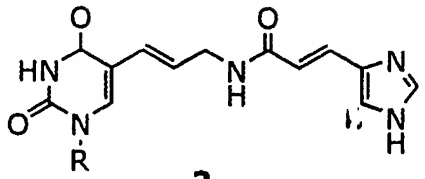


65B


Evolving Plastics
L2
L1 — M — L3
Ln
monomer
random DNA/RNA ligated to metal center
library of potential catalysts; each unique by nature of DNA/RNA appendage
select for desired polymers and consequently, the desired catalysts
i) amplify & further diversify DNA
ii) append to ligand backbone
add monomer and repeat
pool of desired catalyst molecules (which can be amplified by nature of DNA/RNA)
new library of potential catalysts, enriched in selected activity
pool of DNA/RNA-transition metal catalysts evolved towards the selected activity AND their desired polymer products



1



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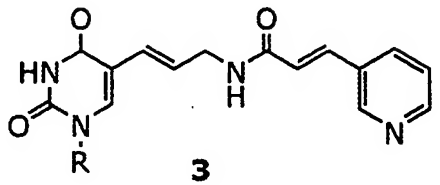

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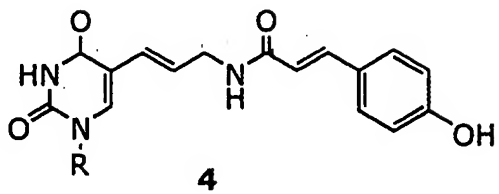
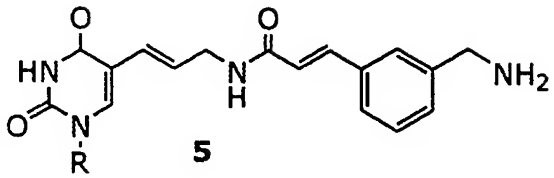


4



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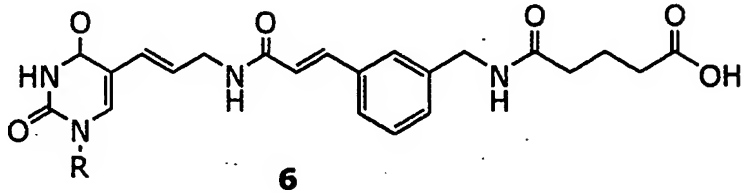

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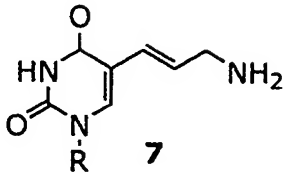

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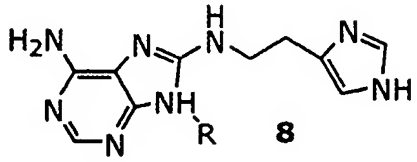

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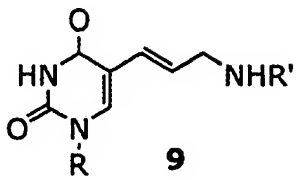
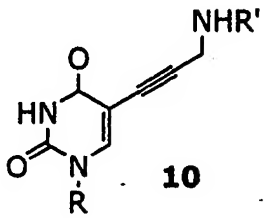


9



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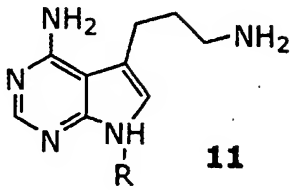
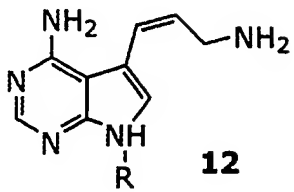


11



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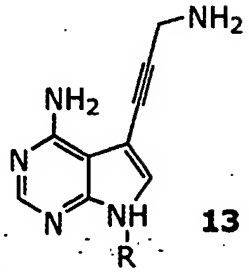
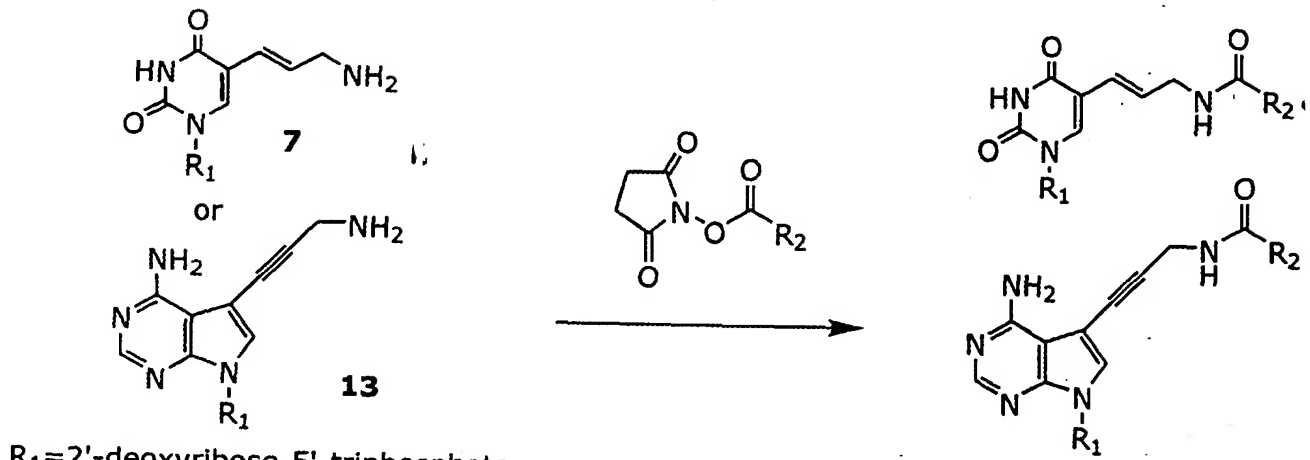


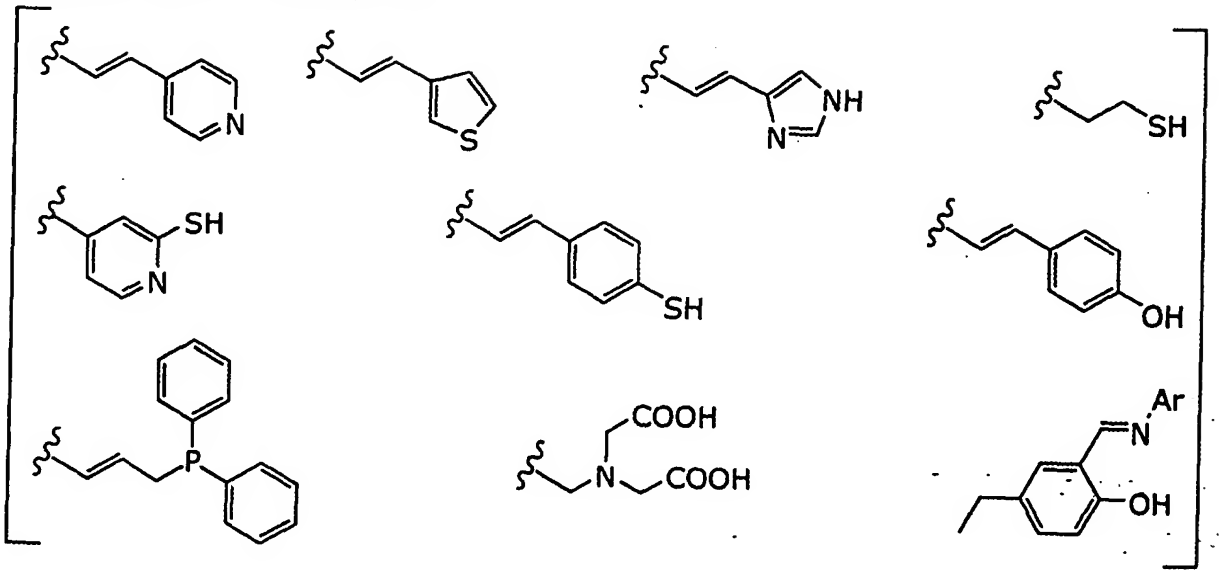
13

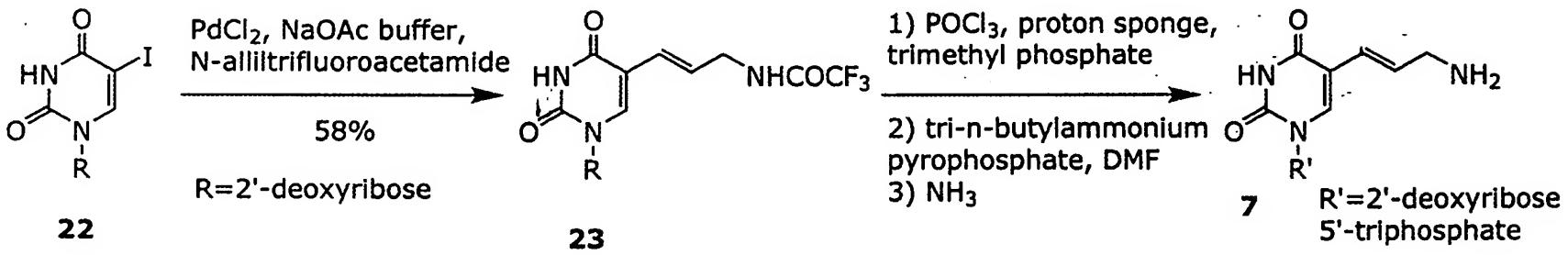
R=2'-deoxyribonuclelotide 5'-triphosphate



R_1=2'-deoxyribose-5'-triphosphate

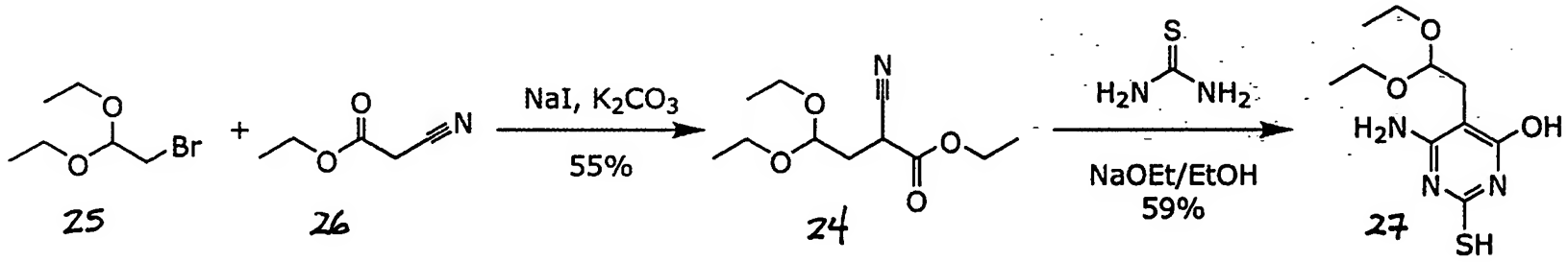
R_2=



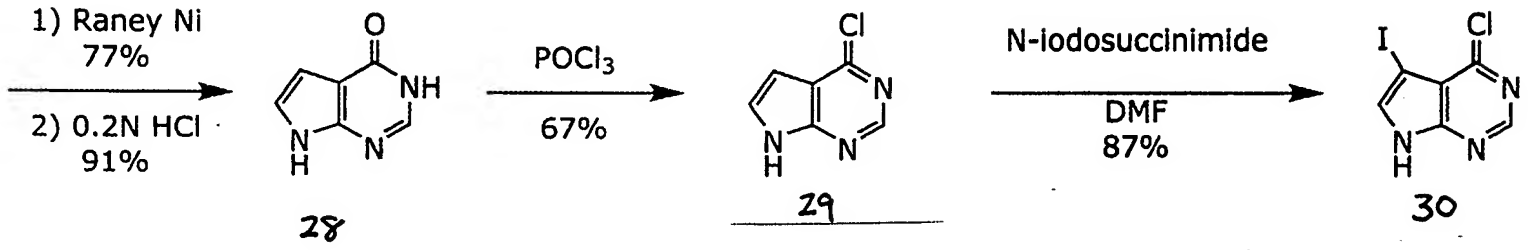


22 23 7

69

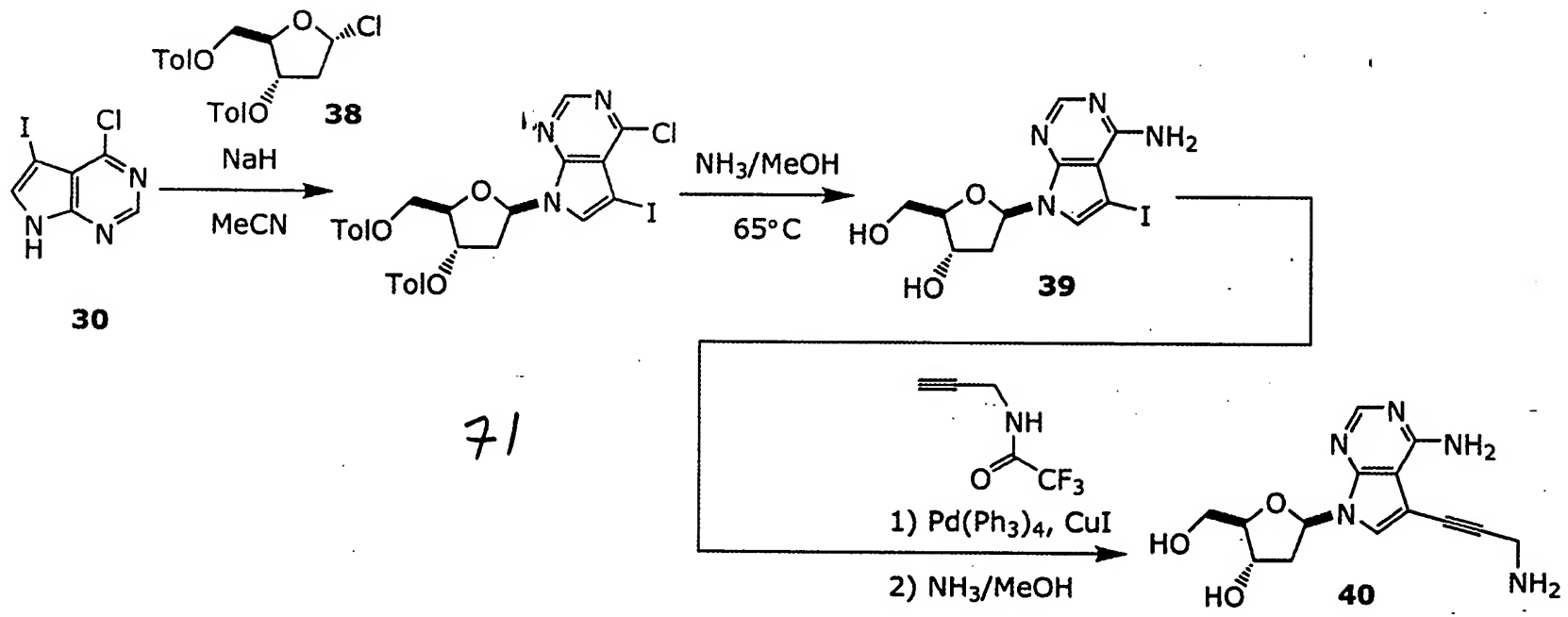


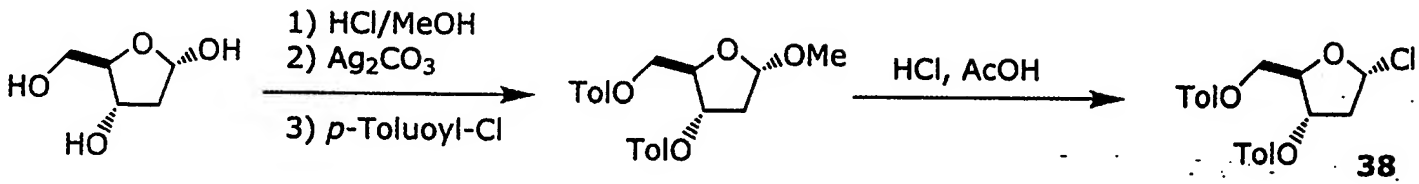
25 26 24 27



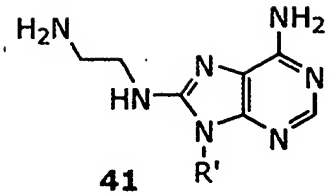
28 29 30

70

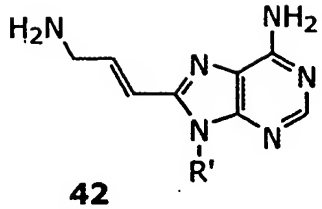

TolO
Cl
TolO
38
I
Cl
N
N
H
30
NaH
MeCN
TolO
TolO
I
Cl
N
N
I
NH3/MeOH
65°C
HO
HO
39
NH2
N
N
N
I
71
NH
O
CF3
1) Pd(Ph3)4, CuI
2) NH3/MeOH
HO
HO
40
NH2
NH2
N
N
N


HO
OH
HO
1) HCl/MeOH
2) Ag2CO3
3) p-Toluoyl-Cl
TolO
OMe
TolO
HCl, AcOH
TolO
Cl
TolO
38

72

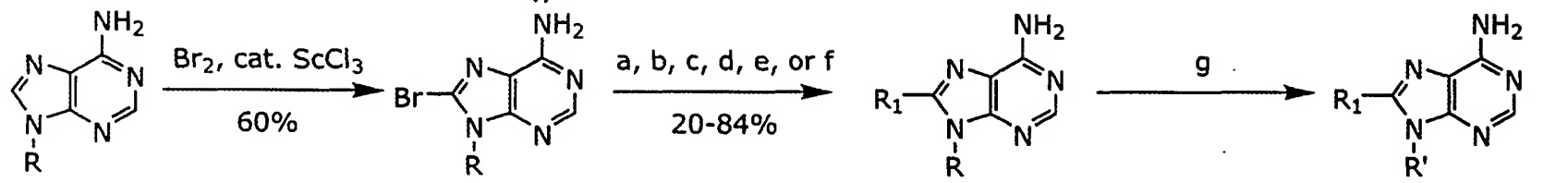


41



42

R' = 2'-deoxyribose-5'-triphosphate

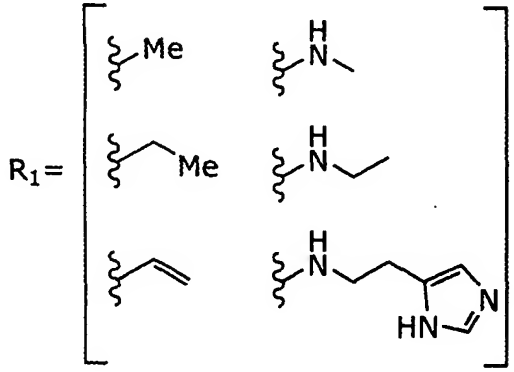


R=2'-deoxyribose

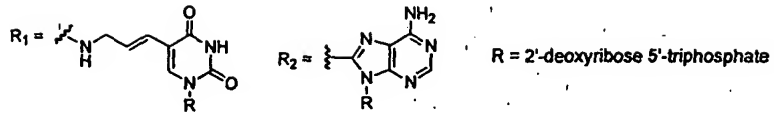
31

32-37

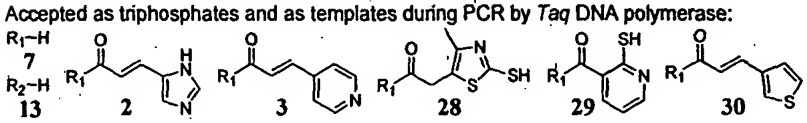
R'=2'-deoxyribose-5'-triphosphate



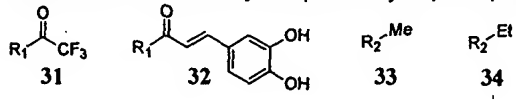
a (R=Me): 1) HMDS, dioxane, 2) Me_4Sn, $Pd(PPh_3)_4$, NMP, 3) K_2CO_3, MeOH
b (R=Et): 1) HMDS, dioxane, 2) Et_4Sn, $Pd(PPh_3)_4$, NMP, 3) K_2CO_3, MeOH
c ($R=CH_2=CH_2$): 1) HMDS, dioxane, 2) $(CH_2=CH)_4Sn$, $Pd(PPh_3)_4$, NMP, 3) K_2CO_3, MeOH
d (R=NHMe): $MeNH_2$, H_2O
e (R=NHEt): $EtNH_2$, H_2O
f (R=histaminyl): histamine, EtOH, heat

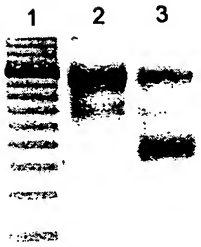


Accepted as triphosphates and as templates during PCR by *Taq* DNA polymerase:

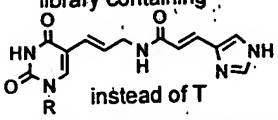


Nucleotides not successfully incorporated by *Taq* DNA polymerase:



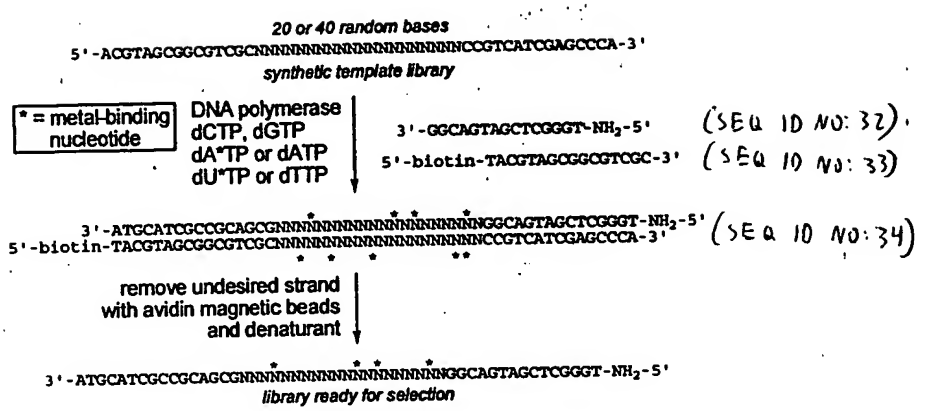


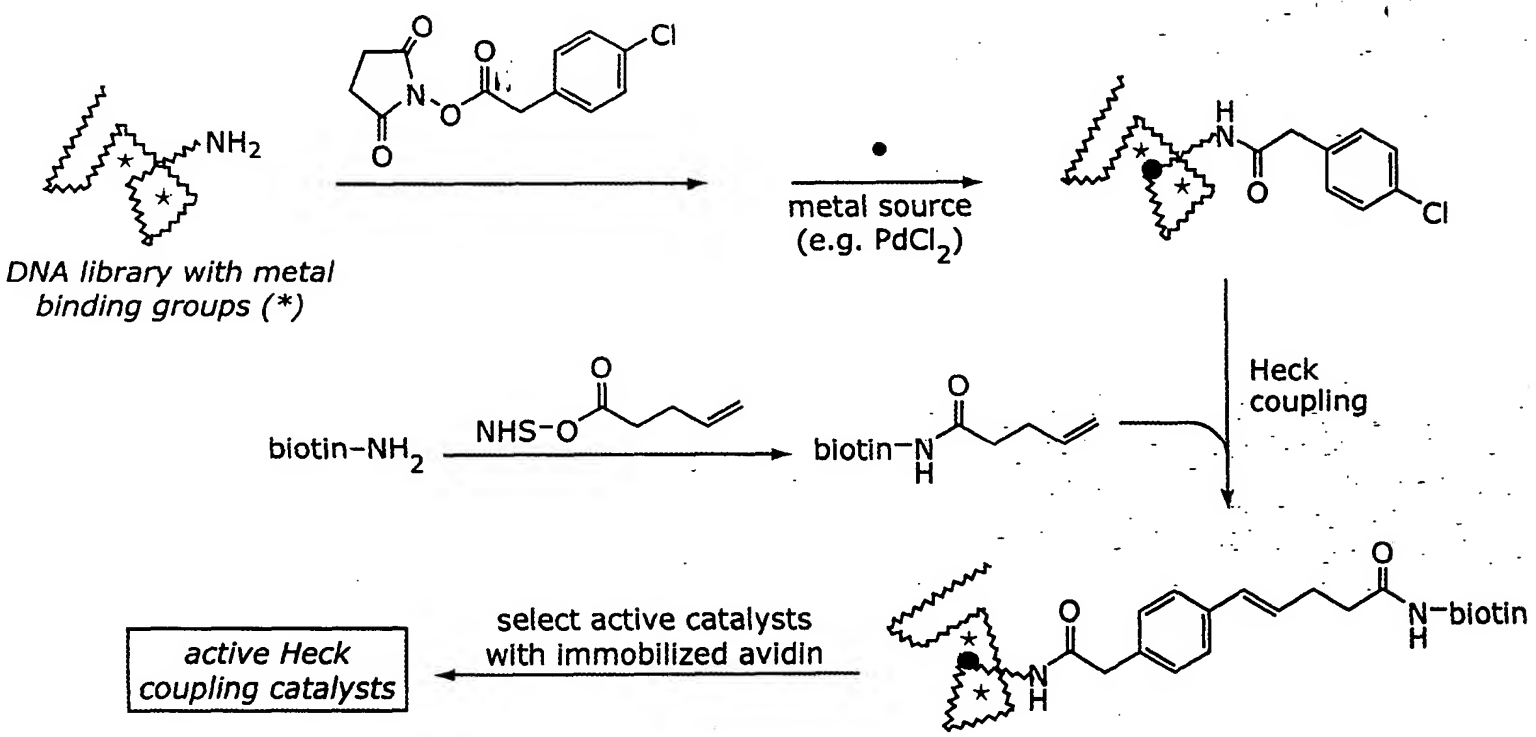
1 = 10 bp ladder
2 = error-prone PCR-generated library containing



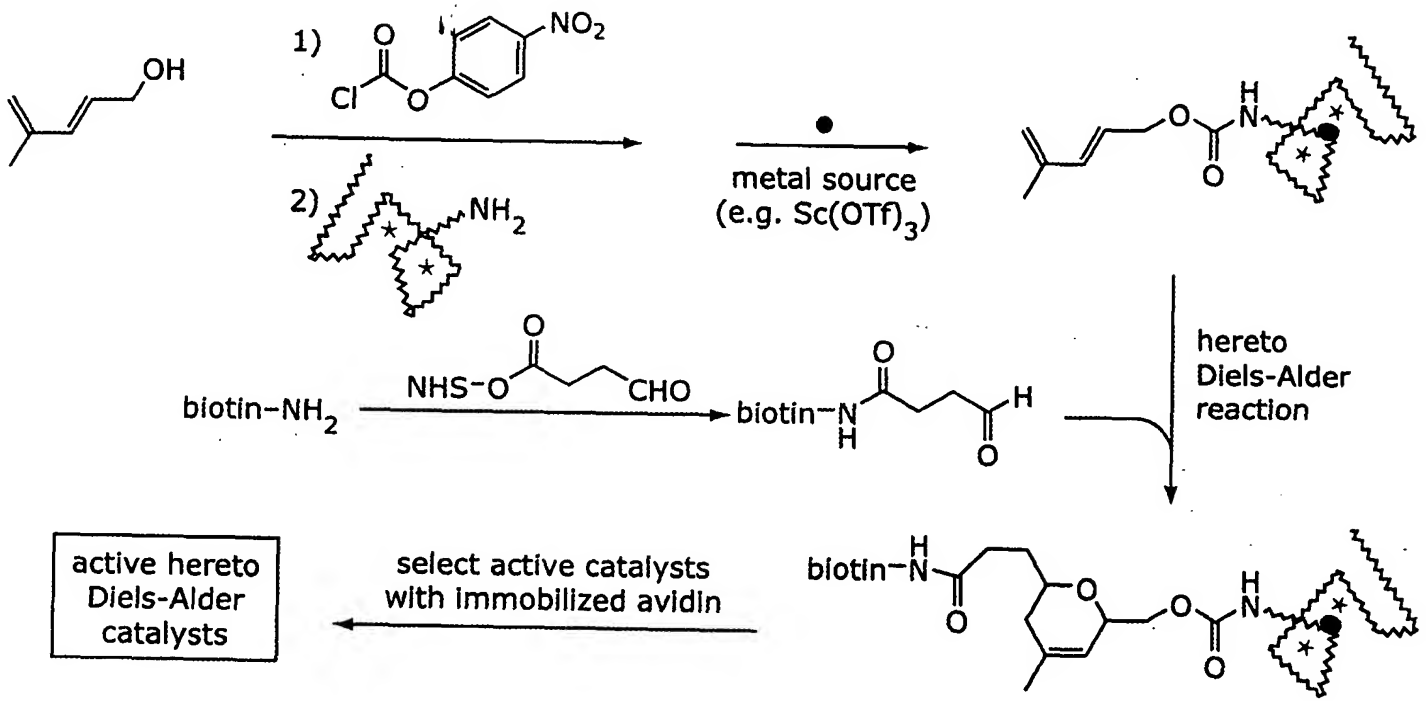
instead of T

3 = lane 2 following purification of the desired strand

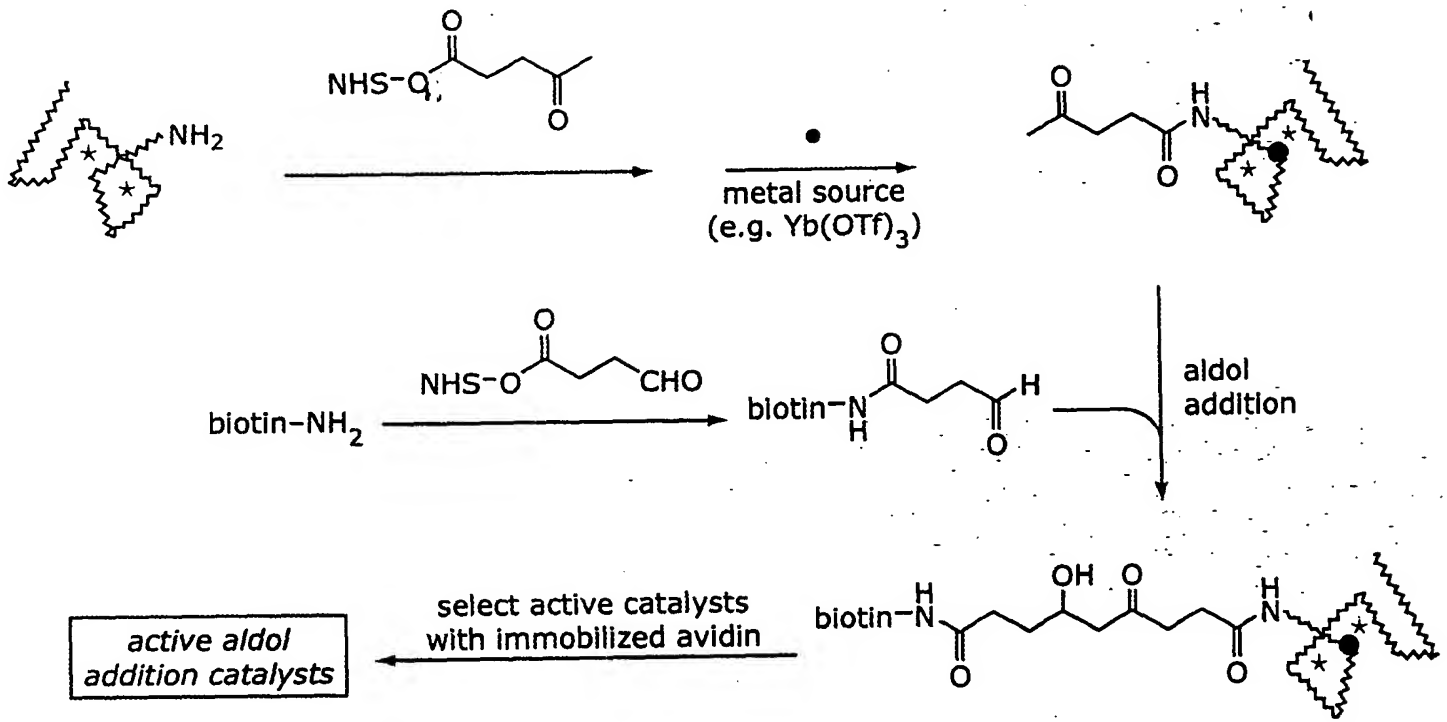

20 or 40 random bases
5'-ACGTAGCGGCGTCGCNNNNNNNNNNNNNNNNNNNNCCGTCATCGAGCCCA-3'
synthetic template library
* = metal-binding nucleotide
DNA polymerase
dCTP, dGTP
dA*TP or dATP
dU*TP or dTTP
3'-GGCAGTAGCTCGGGT-NH2-5' (SEQ ID NO: 32)
5'-biotin-TACGTAGCGGCGTCGC-3' (SEQ ID NO: 33)
3'-ATGCATCGCCGCAGCGNNNNNNNNNNNNNNNNNNNNGGCAGTAGCTCGGGT-NH2-5' (SEQ ID NO: 34)
5'-biotin-TACGTAGCGGCGTCGCNNNNNNNNNNNNNNNNNNNNCCGTCATCGAGCCCA-3'
remove undesired strand with avidin magnetic beads and denaturant
3'-ATGCATCGCCGCAGCGNNNNNNNNNNNNNNNNNNNNGGCAGTAGCTCGGGT-NH2-5'
library ready for selection


NH2
Cl
metal source
(e.g. PdCl2)
DNA library with metal
binding groups (*)
biotin–NH2
NHS–O
Heck
coupling
select active catalysts
with immobilized avidin
active Heck
coupling catalysts
N–biotin

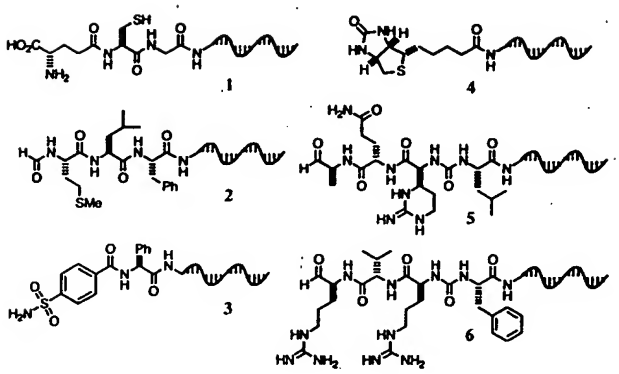
78A


OH
1)
Cl
O
O
NO2
2)
NH2
metal source
(e.g. Sc(OTf)3)
O
H
N
O
hereto
Diels-Alder
reaction
biotin–NH2
NHS–O
O
CHO
biotin–N
H
O
H
O
biotin–N
H
O
O
O
H
N
O
active hereto
Diels-Alder
catalysts
select active catalysts
with immobilized avidin

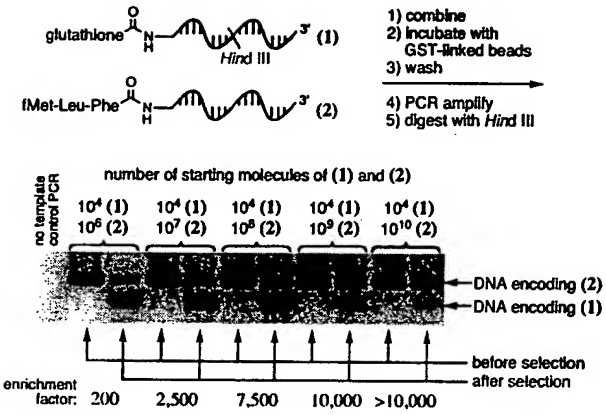
78B

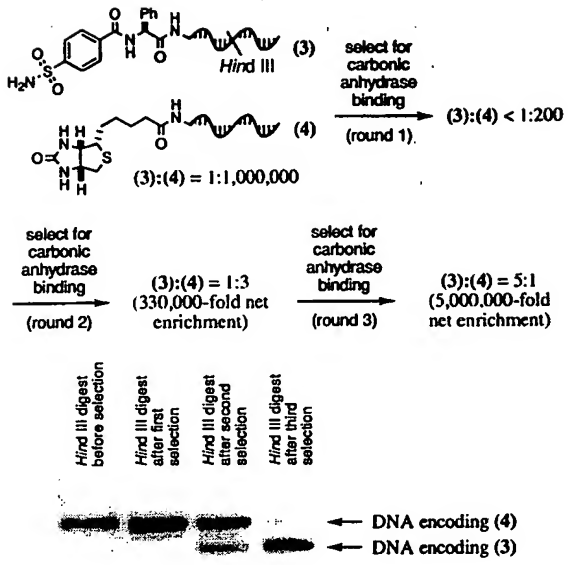

NHS-O
NH2
metal source
(e.g. Yb(OTf)3)
biotin-NH2
NHS-O
CHO
biotin-N
H
aldol
addition
select active catalysts
with immobilized avidin
active aldol
addition catalysts
biotin-N
H
OH
H
N

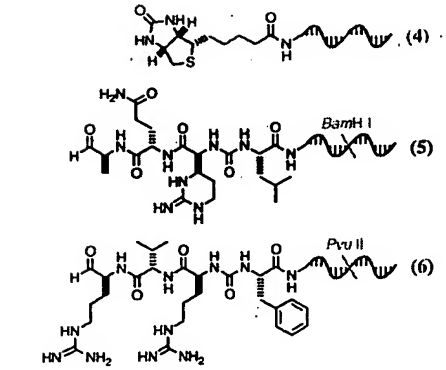
78C

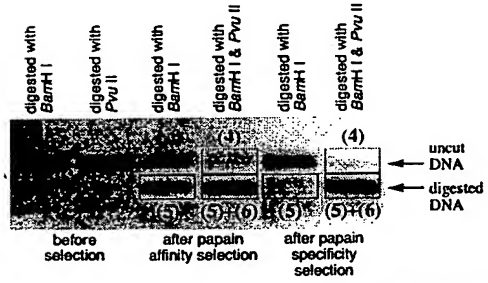


DNA-linked molecule	target protein	predicted activity	enrichment factor	sensitivity (mol)
1	glutathione *S*-transferase	K_d = 10 μM	2,500	10^{-20}
3	carbonic anhydrase	K_d = 0.9 nM	330	10^{-20}
4	streptavidin	K_d = 40 fM	4,400	10^{-18}
5	papain	IC_{50} = 14 μM	64	10^{-16}
5	chymotrypsin	IC_{50} = 290 nM	76	10^{-16}
6	papain	IC_{50} = 270 nM	98	10^{-18}
6	trypsin	K_d = 100 nM	125	10^{-17}

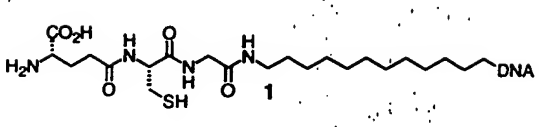

glutathione
3' (1)
Hind III
fMet-Leu-Phe
3' (2)
1) combine
2) incubate with GST-linked beads
3) wash
4) PCR amplify
5) digest with Hind III
number of starting molecules of (1) and (2)
no template control PCR
10⁴ (1) 10⁶ (2)
10⁴ (1) 10⁷ (2)
10⁴ (1) 10⁸ (2)
10⁴ (1) 10⁹ (2)
10⁴ (1) 10¹⁰ (2)
DNA encoding (2)
DNA encoding (1)
before selection
after selection
enrichment factor: 200 2,500 7,500 10,000 >10,000


(3)
HInd III
(4)
select for carbonic anhydrase binding
(round 1)
(3):(4) < 1:200
(3):(4) = 1:1,000,000
select for carbonic anhydrase binding
(round 2)
(3):(4) = 1:3
(330,000-fold net enrichment)
select for carbonic anhydrase binding
(round 3)
(3):(4) = 5:1
(5,000,000-fold net enrichment)
HInd III digest before selection
HInd III digest after first selection
HInd III digest after second selection
HInd III digest after third selection
DNA encoding (4)
DNA encoding (3)

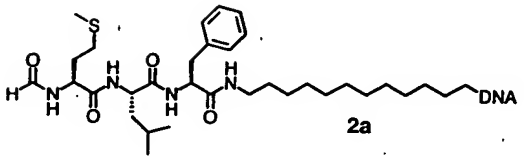




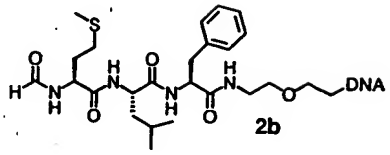
	IC_{50} for chymotrypsin[10c]	IC_{50} for papain[10c]	initial ratio	ratio after papain affinity selection	ratio after papain specificity selection
(4)	>500 μM	>500 μM	24	1	1
(5)	0.29 μM	14 μM	4	12	1
(6)	>500 μM	0.27 μM	1	12	>10



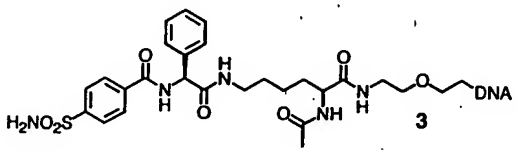
1



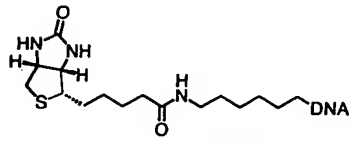
2a



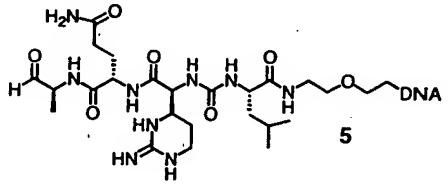
2b



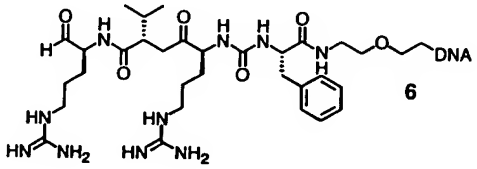
3



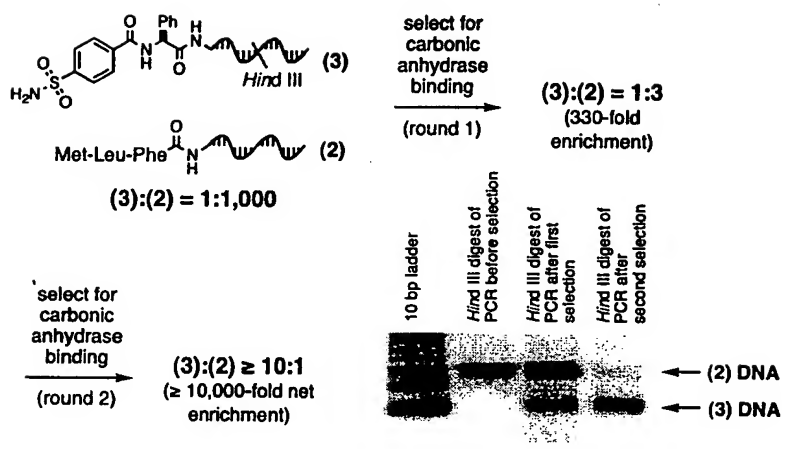
4a, 4b

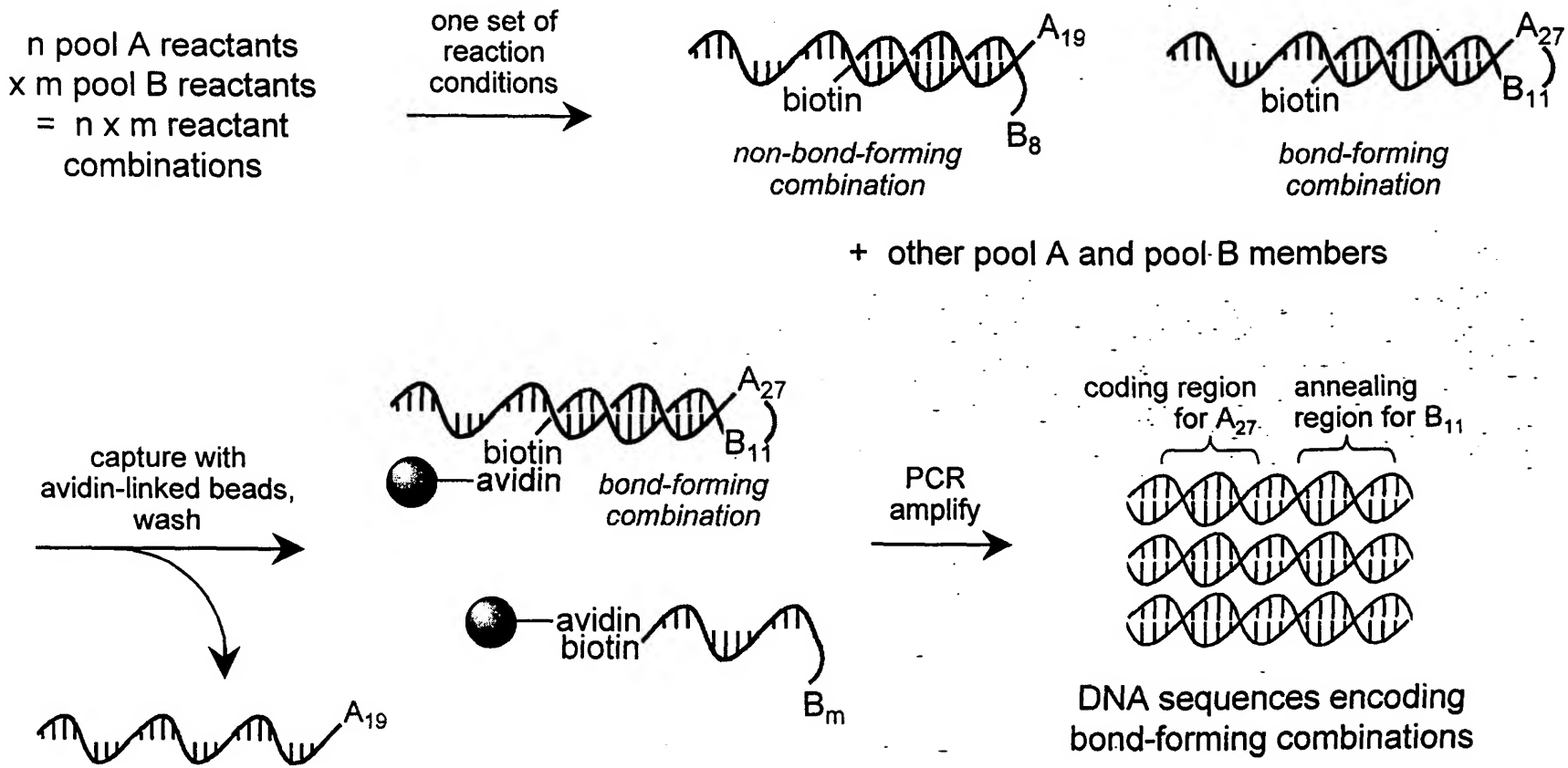


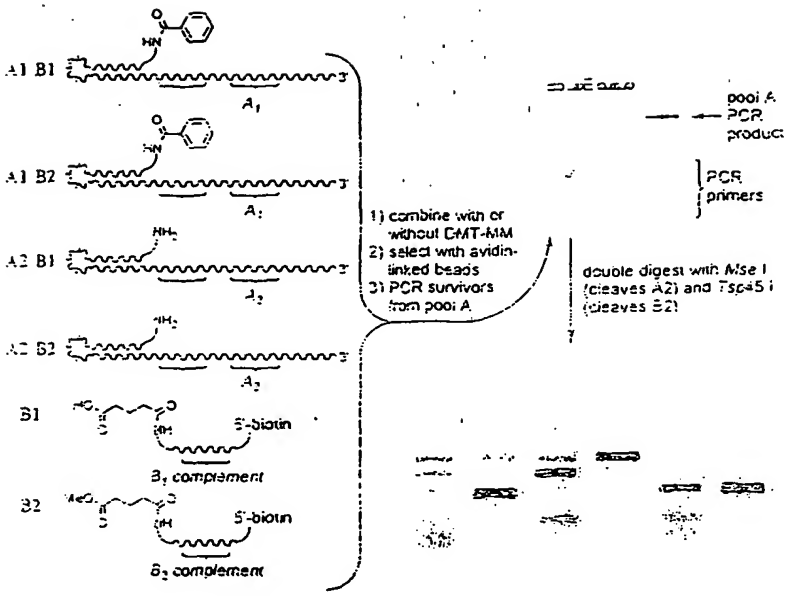
5

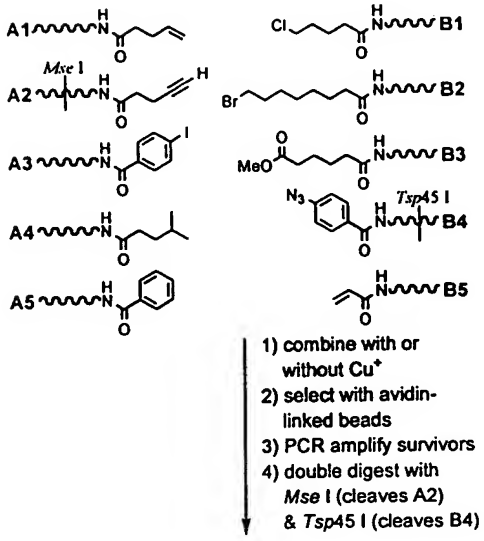


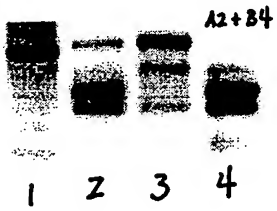
6


(3)
HindIII
(2)
Met-Leu-Phe
(3):(2) = 1:1,000
select for carbonic anhydrase binding
(round 1)
(3):(2) = 1:3
(330-fold enrichment)
select for carbonic anhydrase binding
(round 2)
(3):(2) ≥ 10:1
(≥ 10,000-fold net enrichment)
10 bp ladder
HindIII digest of PCR before selection
HindIII digest of PCR after first selection
HindIII digest of PCR after second selection
(2) DNA
(3) DNA


n pool A reactants
x m pool B reactants
= n x m reactant combinations
one set of reaction conditions
biotin
A_{19}
B_8
non-bond-forming combination
biotin
A_{27}
B_{11}
bond-forming combination
+ other pool A and pool B members
capture with avidin-linked beads, wash
biotin
avidin
A_{27}
B_{11}
bond-forming combination
avidin
biotin
B_m
A_{19}
non-bond-forming combinations
PCR amplify
coding region for A_{27}
annealing region for B_{11}
DNA sequences encoding bond-forming combinations


A1 B1
A1 B2
A2 B1
A2 B2
B1
B2
5'-biotin
5'-biotin
1) combine with or without GMT-MM
2) select with avidin-linked beads
3) PCR survivors from pool A
double digest with MseI (cleaves A2) and TspE5 I (cleaves B2)
pool A PCR product
PCR primers


A1
A2
Mse I
A3
A4
A5
Cl
B1
Br
B2
MeO
B3
N3
*Tsp*45 I
B4
B5
1) combine with or without Cu+
2) select with avidin-linked beads
3) PCR amplify survivors
4) double digest with *Mse* I (cleaves A2) & *Tsp*45 I (cleaves B4)


A2+B4
1
2
3
4

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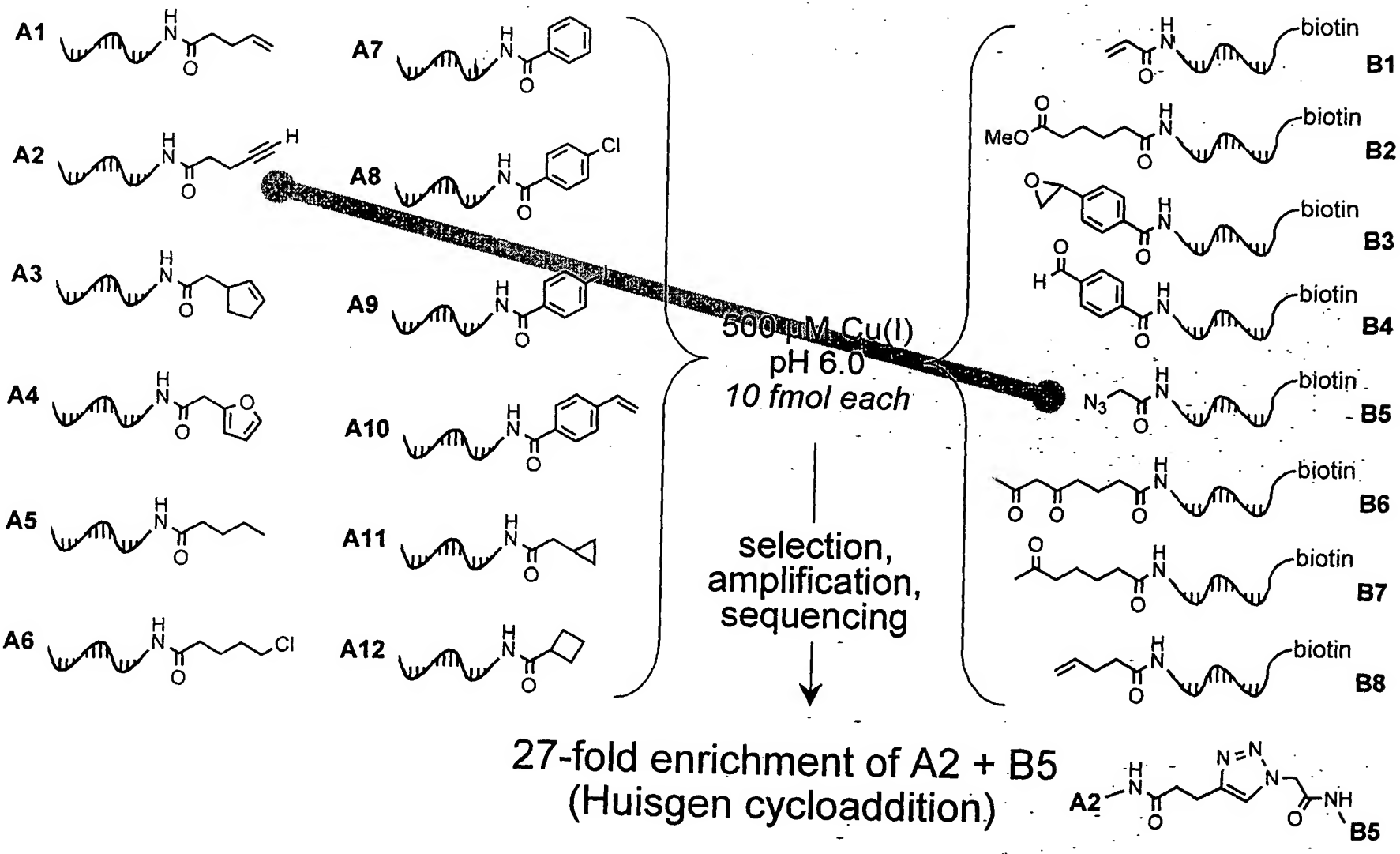
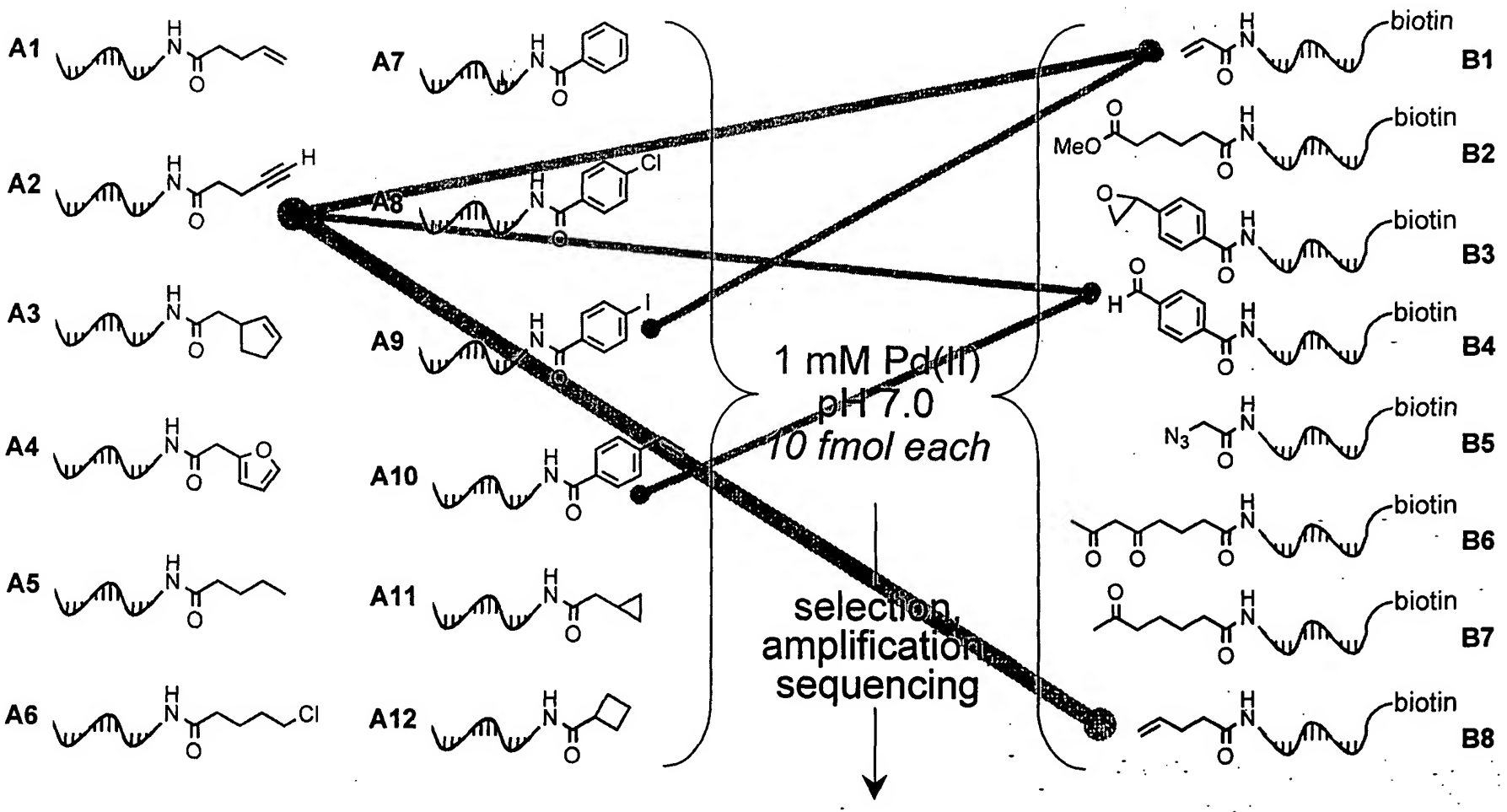
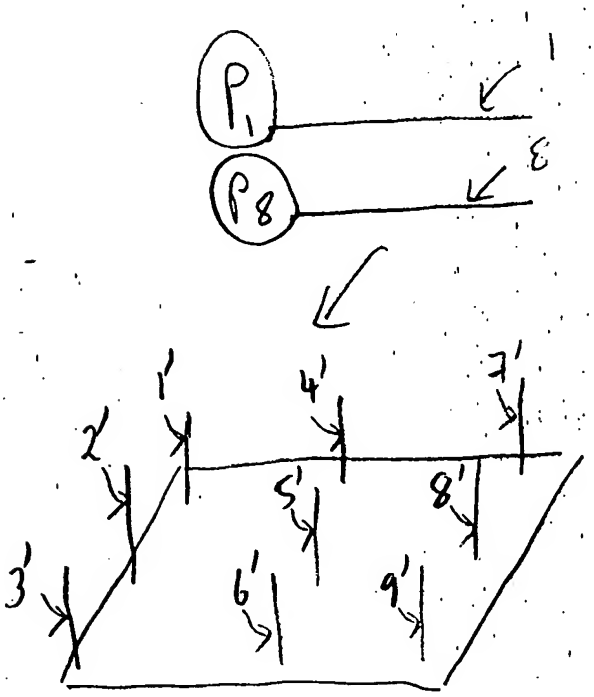

A1
A2
A3
A4
A5
A6
A7
A8
A9
A10
A11
A12
500 µM Cu(I)
pH 6.0
10 fmol each
selection,
amplification,
sequencing
biotin
B1
B2
B3
B4
B5
B6
B7
B8
27-fold enrichment of A2 + B5
(Huisgen cycloaddition)


A1
A2
A3
A4
A5
A6
A7
A8
A9
A10
A11
A12
1 mM Pd(II)
pH 7.0
10 fmol each
selection,
amplification,
sequencing
biotin
B1
B2
B3
B4
B5
B6
B7
B8



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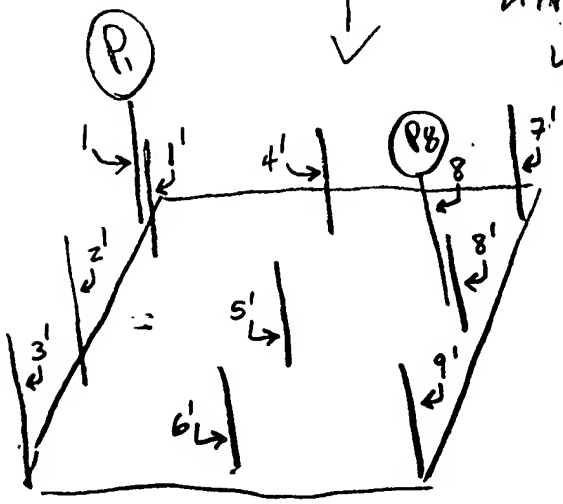
Combine under annealing conditions & wash away unbound material



Detect bound P1 and P8

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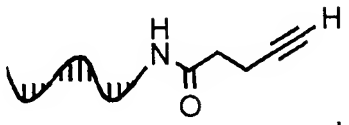
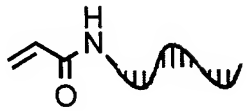
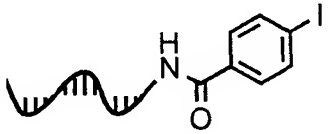
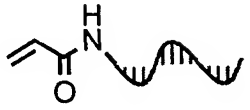
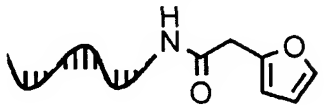
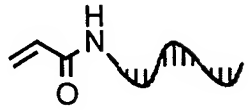
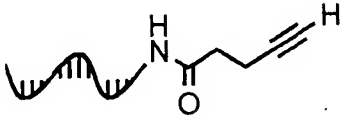
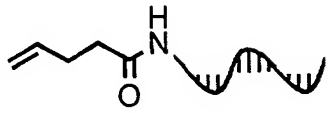
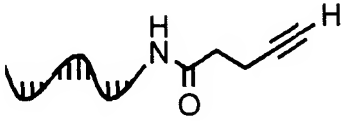
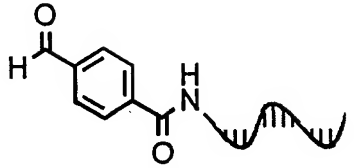
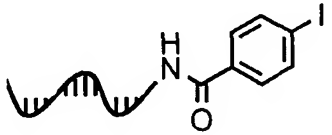
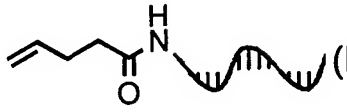
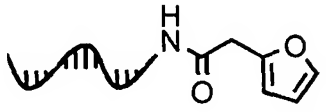
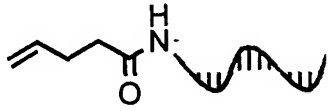
				array signal ÷ background	DNA-templated reaction yield
	+			78-fold	75-95%
	+		(Heck)	76-fold	71-91%
	+			56-fold	70-90%
	+			44-fold	75-95%
	+			38-fold	53-73%
	+		(Heck)	30-fold	57-77%
	+			22-fold	75-95%